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Edited by G. C. Mead

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Introduction

Access to a nutritionally adequate and safe food supply has long been regarded as a basic human right or, at least, an aspiration. Among the foods capable of meeting such a need, poultry products, including eggs, have a highly important part to play throughout the world. In developed countries, consumption of poultry meat has continued to rise, as consumers recognise the nutritious, low-fat attributes of the meat itself and the wide range of value-added products now available that provide choice, convenience and flexibility in meal preparation. Also, the technical efficiency and economies of scale in commercial production and processing of poultry have helped to ensure that product prices remain competitive in the marketplace.

Despite the obvious benefits to consumers, poultry products are not exempt from certain public health concerns that continue to affect the food supply chain as a whole and foods of animal origin in particular. These are due to the possible presence of specific disease agents, whether biological or chemical, that can have a wide range of consequences for human health. The situation is often exacerbated by the adverse publicity that such problems attract. Hardly a week goes by without some kind of food 'scare' being reported in the press, and poultry products have had more than their fair share of attention in this respect. More recent examples from around the world include *Salmonella* in eggs, dioxin contamination of chicken meat and *Listeria* in ready-to-eat poultry products. Inevitably, these issues raise questions about the adequacy of present controls and result in political pressure to take any corrective action that is perceived to be necessary. The bad publicity can have a devastating effect on the industry, since consumers simply stop buying the suspect product. The power of the consumer is also evident in the way that retailers respond to consumer concerns as a means of positioning themselves against their competitors.

In the major poultry-producing countries, production systems tend to be highly integrated, which means that a single company may carry out the whole gamut of relevant activities, from hatching of chicks, through feed production and rearing of breeders and broilers, to carcass processing and product manufacture. Thus, food safety control is facilitated at all stages of the supply chain, but how is it best achieved? Over the years, a substantial body of knowledge has accumulated on the health hazards associated with poultry products and their control. This has involved developments in a variety of fields, including food science and technology, veterinary science, animal husbandry, microbiology and analytical chemistry, to name but a few. However, making best use of the available knowledge requires a continuing input from individuals such a research scientists, public health officials, legislators and technical personnel in industry.

The present book provides a unique compilation of food safety topics that are relevant to the production and processing of poultry meat and eggs, and it brings together contributions from acknowledged experts in a number of different countries. The main hazards, whether biological or chemical, are discussed in detail, as are many of the current methods for their detection and quantification, and the 'farm-to-fork' approach that is generally taken in establishing suitable control strategies. Of particular relevance here are recent developments in Quantitative Risk Assessment and the application of Hazard Analysis Critical Control Point principles throughout the poultry supply chain. Also included is an up-to-date account of *Salmonella* control in Swedish poultry production – widely recognised as a 'gold standard', but controversial in relation to its applicability in other countries, outside Scandinavia. In view of the well-known difficulties in excluding foodborne pathogens from live poultry, particular attention is given to possible means of decontaminating eggs or processed carcasses. Some methods, such as the use of bacteriophage or bacteriocins for treating carcasses, are very new and still under development. Others, including low-dose irradiation, are already supported by a large amount of research evidence, and their suitability is discussed fully. Finally, the book pays due regard to the role of consumer handling in the microbiological hazards that can arise when eggs or meat are prepared for consumption in the kitchen. Overall, the volume contains a large amount of information on key aspects of food safety control that are so vital to the continued success of the worldwide poultry industry. It thereby serves as a reference book in this respect and covers the needs of poultry science students and all those that seek authoritative information on such an important subject.

As the Editor, I should like to thank each of the other contributors to the book for their hard work and dedication. Clearly, a book of this kind is only as good as the individual authors, but their combined expertise has resulted in a work that appears to have no rival in other, current publications. Above all, I am grateful to the Publishers for their support and friendly collaboration, and to my wife, Val, for her indispensable help and understanding throughout the book's gestation period.

G. C. Mead

1

Bacterial infection of eggs

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1.1 Introduction

Although a variety of microbes, including pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes* and *Yersinia enterocolitica,* are occasionally found on egg shells and are capable of surviving or growing in egg contents (Burley and Vadehra, 1989; Board and Fuller, 1994; Stadelman and Cotterill, 1995; Ricke *et al.*, 2001), the history of eggs as a source of human illness has almost exclusively concerned bacteria of the genus *Salmonella*. Until the late 1960s, human salmonellosis (involving a diversity of serotypes) was commonly attributed to table eggs with cracked or dirty shells or to egg products that had not been heated sufficiently during processing to completely destroy pathogens. In the USA, the 1970 Egg Products Inspection Act prohibited the sale of cracked and dirty table eggs and mandated reliably effective pasteurization standards for liquid egg products, thereby leading to a very dramatic reduction in the frequency with which human illness was linked to eggs in the years that followed. However, by the mid-1980s, a newly-emerging public health issue again focused attention on eggs as a source of *Salmonella* transmission (St Louis *et al.*, 1988). In this, more recent version of the story of eggs and *Salmonella*, human illness was associated primarily with clean and intact, Grade A table eggs. Moreover, the vast majority of these disease outbreaks involved a single serotype, *S. enterica* serovar Enteritidis (*S.* Enteritidis). An international surge in human *S.* Enteritidis (SE) infections has been principally connected to contaminated eggs (Angulo and Swerdlow, 1999; van de Giessen *et al.*, 1999; Wall and Ward, 1999). In the USA, approximately 80% of the human SE outbreaks for which a food source could be identified have been attributed to eggs or egg-containing foods (Patrick *et al.*, 2004). Accordingly, developing and

implementing effective programmes to diminish the likelihood that consumers will be exposed to contaminated eggs has become an important objective for both government and industry on several continents (Hogue *et al.*, 1997b; Cogan and Humphrey, 2003).

This chapter will explore the causes, characteristics, consequences and control of SE contamination of commercially-produced eggs. Section 1.2 discusses the routes of SE transmission into poultry flocks and into eggs, including the host and bacterial factors that promote the infection of laying hens and how these infections bring about *Salmonella* deposition inside eggs. Section 1.3 discusses the nature of SE contamination of eggs, including the deposition, survival and multiplication of the pathogen in various locations inside eggs. Section 1.4 discusses and assesses the most promising approaches to achieving sustainable, long-term reductions in egg-associated human illness.

1.2 Routes of transmission of *Salmonella* into poultry flocks and eggs

1.2.1 External and internal contamination of eggs

Although extensive microbial contamination of egg shells is uncommon at the time of oviposition, avian faecal material and other environmental sources in the laying house can rapidly introduce bacteria onto eggs (Board and Fuller, 1994). Inadequate sanitation in egg processing facilities is another possible cause of shell contamination (Davies and Breslin, 2003a). If not removed during processing, pathogens on the shell surface can be transferred to the edible, liquid portion of the egg, when the shell is broken to release the contents for use or consumption. Moreover, bacteria can also penetrate through shells to reach the contents. The porous shell is not a significant obstacle to bacterial penetration, although the underlying shell membranes are a more effective barrier (Burley and Vadehra, 1989; Ricke *et al.*, 2001). Eggs are routinely washed in some countries to remove pathogens and spoilage organisms from shells, but improper control of temperature during egg washing can lead to a pressure gradient that promotes the movement of microbes through the shell membranes and into the contents (Stadelman and Cotterill, 1995).

Diverse *Salmonella* serotypes are found on shells, but only SE has been associated with a large number of egg-transmitted, human disease outbreaks in recent decades. A Japanese study reported that SE was the only one of six serotypes tested that was deposited in egg yolks by experimentally-infected hens (Okamura *et al.*, 2001a). Similarly, a study in the UK found SE only inside naturally-contaminated eggs, even though a wide assortment of serotypes was present on the shells of these eggs (Humphrey *et al.*, 1991b). This suggests that some mechanism other than shell contamination is responsible for the current public health problems related to eggs. This other process, often referred to somewhat misleadingly as ‘transovarian transmission’, is the consequence of systemic infection of laying hens with *Salmonella* that results in deposition of

the pathogen inside the contents of developing eggs in the reproductive tract (Timoney *et al.*, 1989; Gast and Beard, 1990a).

1.2.2 Systemic infection of hens and transovarian transmission of *Salmonella* Enteritidis

Like most other paratyphoid (non-host-adapted) *Salmonella* serotypes, SE is usually introduced to chickens via the gastrointestinal tract. After oral ingestion from the environment, SE colonizes several regions of the tract, particularly the crop and caeca (Turnbull and Snoeyenbos, 1974). Invasion through mucosal epithelial cells can then lead to systemic dissemination to a wide array of internal organs, including reproductive tissues (Gast and Beard, 1990b; Humphrey *et al.*, 1993). By colonizing the ovary (the site of yolk maturation and release) and the oviduct (the site of albumen secretion around the descending yolk), SE appears to gain access to the contents of eggs (Miyamoto *et al.*, 1997; Okamura *et al.*, 2001a; De Buck *et al.*, 2004). Some investigators have found SE inside pre-ovulatory follicles and in developing eggs removed from the oviducts of infected hens before oviposition (Thiagarajan *et al.*, 1994; Keller *et al.*, 1995). Recent reports have also implicated *S.* Heidelberg as an egg-transmitted pathogen (Hennessy *et al.*, 2004), and an experimental-infection study documented the ability of some strains of this serotype to colonize reproductive tissues and be deposited inside eggs (Gast *et al.*, 2004).

In experimental infection studies, laying hens have typically produced internally-contaminated eggs for only a few weeks following oral inoculation (Gast and Beard, 1990a; Gast and Holt, 2000a). However, in commercial laying flocks, the patterns of egg contamination over time are far more irregular, as infection spreads gradually through each house. Contamination of eggs with SE seems to be a generally infrequent phenomenon within infected flocks. Two studies of environmentally-positive, commercial laying flocks in the USA have indicated a prevalence of contaminated eggs of less than 0.03% (Kinde *et al.*, 1996; Henzler *et al.*, 1998). The overall incidence of SE contamination of eggs from commercial flocks in the USA has been estimated at around 0.005% (Ebel and Schlosser, 2000). Likewise, egg contamination usually occurs at relatively low frequencies in experimental infection studies, even after the administration of very large oral doses of SE to laying hens (Humphrey *et al.*, 1991a; Gast and Holt, 2001a; Gast *et al.*, 2002).

1.2.3 Sources of introduction of *Salmonella* Enteritidis into poultry flocks

A recent national survey in the USA indicated that approximately 7% of the commercial laying flocks in that country were environmentally positive for SE (Garber *et al.*, 2003). The leading potential sources that can introduce SE into laying flocks are the replacement chicks themselves, the poultry house environment, rodents and other pests, and feed. Hatcheries, too, are significant because of the combined circumstances of possible vertical transmission of SE

from infected breeder flocks (Methner *et al.*, 1995; Berchieri *et al.*, 2001), the especially high susceptibility of newly hatched chicks to bacterial colonization of the intestinal tract (Duchet-Suchaux *et al.*, 1995; Gast and Benson, 1996), and the extensive circulation of contaminated dust and aerosols in the crowded conditions within hatcher cabinets (Davies *et al.*, 2001; Mitchell *et al.*, 2002).

Even if not exposed to SE as chicks or growing pullets, laying hens can still be infected subsequently with the pathogen, if transferred into a laying house that was not adequately decontaminated after removal of a previous, infected flock. A Dutch study (van de Giessen *et al.*, 1994) reported that most commercial flocks became infected for the first time as a result of environmental exposure to contaminated laying houses. A large field study in the USA (Schlosser *et al.*, 1999) showed that the presence of SE in laying house environments was strongly correlated with the probability that flocks would produce contaminated eggs. Environmental reservoirs of SE have sometimes been found to persist in laying houses, even after intensive cleaning and disinfection is applied upon termination of a flock (Davies and Wray, 1996; Davies and Breslin, 2003c). In one study, SE could still be isolated from litter, dried faeces and feed for 26 months after removal of the chickens (Davies and Breslin, 2003d). Even after effective cleaning and disinfection, pests such as mice can re-introduce SE into poultry farms (Davies and Wray, 1995a).

An extremely diverse assortment of vectors, including insects, reptiles, wild birds, rodents, livestock, pets and humans, can all transmit SE to poultry, their housing environment, or their feed and water sources. Insects, particularly beetles (Gray *et al.*, 1999) and flies (Olsen and Hammack, 2000), are common in poultry houses and can carry SE, both externally and internally. Mice have been the most consistent and convincing documented source of SE for contaminating poultry facilities. Environmental contamination with SE has often correlated directly with heavy mouse infestations (Henzler and Opitz, 1992; Schlosser *et al.*, 1999). Mice captured on poultry farms have been infected with SE at high frequencies and the droppings have been shown to be capable of transmitting the organism to chickens (Davies and Wray, 1995b; Guard-Petter *et al.*, 1997). Moreover, the use of molecular finger-printing has linked clones of SE found in mice, laying hens and eggs (Liebana *et al.*, 2003).

Feed is always a possible source of *Salmonella*, because of both the presence of the organisms in feed ingredients and the occurrence of reservoirs of contamination in feed mills (Davies and Wray, 1997; Whyte *et al.*, 2003; Jones and Richardson, 2004). However, actual epidemiological links between poultry feedstuffs and SE infections in either laying flocks or humans have been very infrequent (Poppe *et al.*, 1991; Veldman *et al.*, 1995). Nevertheless, in a Japanese study, serological and molecular typing connected isolates from feed and egg contents (Shirota *et al.*, 2001).

Once SE is introduced into a poultry house, environmental and management conditions can promote further distribution of the pathogen throughout the flock. In particular, airborne circulation of contaminated dust particles and aerosols can disseminate bacteria very widely (Nakamura *et al.*, 1997; Gast *et al.*, 1998;

Holt *et al.*, 1998). Reduction of circulating, airborne particulates by an electrostatic space-charge (negative air ionization) system has been reported to reduce the transmission of *Salmonella* infection to chicks under experimental conditions (Gast *et al.*, 1999; Mitchell *et al.*, 2002). Insect and rodent vectors, human activity and poultry house equipment can also transport bacterial pathogens within laying flocks.

1.2.4 Host and bacterial factors that promote *Salmonella* Enteritidis infection in poultry and egg contamination

Differences in the susceptibility of chickens to SE infection can lead to corresponding differences in the likelihood that contaminated eggs will be produced. One parameter that has considerable influence on the susceptibility of chickens to *Salmonella* is their age. Newly hatched chicks lack a complete gastrointestinal microflora to serve as a protective barrier against colonization by pathogens (Stavric *et al.*, 1987) and, accordingly, are highly susceptible to infection. Large oral doses of SE can be lethal for one-day-old chicks (Gast and Benson, 1995), but mortality is much less common when chicks are infected at one week of age or more (Duchet-Suchaux *et al.*, 1995). Infection of very young poultry can also lead to highly persistent intestinal colonization. After experimental exposure of chicks to SE during the first few days of life, the pathogen can persist in the intestinal tracts of many birds for six months or more (Phillips and Opitz, 1995; Gast and Holt, 1998a).

Another issue with significance for the outcome of SE infections concerns the role of genetically-based differences in susceptibility between various lines of chickens. These lines have been reported to differ in the observed frequencies of mortality, organ invasion and egg contamination, following SE inoculation of the live birds (Beaumont *et al.*, 1994; Protais *et al.*, 1996). Differences between lines have also been observed in resistance to persistent intestinal colonization by SE (Beaumont *et al.*, 1999; Berchieri *et al.*, 2001). However, the mechanisms that are responsible for these genetic differences in susceptibility remain incompletely characterized.

A poultry management practice that affects host susceptibility to SE is the use of induced molting by feed deprivation to extend the productive lives of commercial egg-laying flocks. Feed deprivation has been found to increase faecal shedding of SE (Holt and Porter, 1992) and invasion of internal organs (Holt *et al.*, 1995) in orally inoculated hens. Moreover, induced molting can reduce the oral dose of SE needed to establish intestinal colonization (Holt, 1993) and increase the frequency of horizontal transmission between hens (Holt, 1995). Inducing molting by feeding low-nutrient-density substances, such as wheat middlings, has been shown to have significantly less effect on the course of SE infections than does feed deprivation (Seo *et al.*, 2001).

Several bacterial attributes appear to be relevant to determining whether SE will be deposited in eggs laid by infected chickens. The ability to cause egg contamination in experimentally-infected hens has been shown to vary among

SE strains (Gast and Holt, 2000a, 2001c). The expression of potential virulence factors, including flagella, fimbriae, outer membrane proteins and iron-uptake systems, can be influenced by pH and temperature conditions, or by growth in chicken tissues (Chart *et al.*, 1993; McDermid *et al.*, 1996; Walker *et al.*, 1999). Serial *in vivo* passage of an SE isolate through reproductive tissues of groups of laying hens has led to an increase in its frequency of deposition in eggs (Gast *et al.*, 2003). Phenotypic properties, such as growth to high cell densities and the expression of high molecular mass lipopolysaccharides have also been linked to egg contamination (Guard-Petter, 1998, 2001). Multiple microbial attributes, such as the abilities to invade beyond the intestinal tract and to colonize reproductive tissues, may complement each other to produce egg contamination (Guard-Petter, 2001; Gast *et al.*, 2002).

1.3 Characteristics of *Salmonella* contamination of eggs

1.3.1 Deposition of *Salmonella* Enteritidis in eggs: quantity and location

Naturally-contaminated eggs have usually been found to harbor very small numbers of SE cells when tested at short intervals following oviposition. Typically, fewer than ten SE cells are present in each contaminated egg (Humphrey *et al.*, 1989), although much larger bacterial populations have been observed in a small proportion of eggs (Humphrey *et al.*, 1991b). Even after the administration of extremely high oral doses of SE (sometimes as many as 10^9 cells) to hens in experimental infection studies, relatively small numbers of contaminants are generally found in the contents of freshly laid eggs (Gast and Beard, 1992). In one such study, most of the eggs from inoculated hens contained less than one SE cell per ml of liquid egg contents, and none contained more than 67 cells per ml (Gast and Holt, 2000a).

Experimentally infected hens have been reported to deposit SE in either (or sometimes both) the yolk or albumen of developing eggs (Humphrey *et al.*, 1989, 1991b; Gast and Beard, 1990a; Bichler *et al.*, 1996; Gast and Holt, 2000a), perhaps as a consequence of the colonization of different regions of the reproductive tract (ovary or oviduct). Intensive microbiological examination of the yolks of eggs laid by experimentally inoculated hens has indicated that SE is deposited far more frequently in association with the vitelline membrane than inside the yolk contents (Gast and Beard, 1990a; Gast and Holt, 2001a). The predominant perspective on naturally occurring contamination of eggs is that SE is initially deposited more often in the albumen (or at least outside the vitelline membrane) than in the yolk (Humphrey, 1994). This point of view is supported by the relatively small numbers of bacteria that are normally detected inside fresh eggs, because more rapid microbial multiplication to higher numbers would be expected in the nutrient-rich yolk than in the growth-restricting conditions of the albumen. Risk assessment efforts in the USA, conducted to provide an analytical foundation for the development of regulatory responses to control the transmission of SE by eggs, have been built around the assumption

that egg contamination is most often initiated by deposition of the pathogen in the albumen or on the vitelline membrane (Hope *et al.*, 2002; Latimer *et al.*, 2002).

1.3.2 Survival and multiplication of *Salmonella* Enteritidis in albumen and yolk

The avian egg has numerous physical and biochemical barriers to microbial growth that are intended to protect the developing embryo from exposure to pathogens (Burley and Vadehra, 1989; Board and Fuller, 1994; Stadelman and Cotterill, 1995). Although the egg shell itself is rather porous, it is coated with a proteinaceous cuticle and has two underlying shell membranes to provide additional resistance to bacterial penetration. Nevertheless, *Salmonella* and other bacteria are able to move through the external structures of the egg, especially at the large end, where the shell membranes separate to form an air cell (Berrang *et al.*, 1999). The creation of negative pressure inside eggs, when the contents contract during cooling, and the presence of moisture and faecal matter on the shell can promote bacterial penetration into eggs (Berrang *et al.*, 1999). Inside the egg, several components of the albumen are directly or indirectly antimicrobial (Ricke *et al.*, 2001; see also Table 1.1). The most significant of these antibacterial albumen proteins is ovotransferrin, which binds iron to limit its availability to support microbial growth (Baron *et al.*, 1997). Furthermore, the pH of albumen increases as it ages and thereby becomes more inhibitory to bacterial multiplication (see Table 1.2).

Despite this considerable array of protective constituents, SE is able to survive and sometimes even grow slowly in albumen. Several investigators have reported that SE inoculated into separated albumen was able to persist during incubation at warm temperatures for days or even weeks (Lock and Board, 1992; Gast and Holt, 2000b, 2001b), although a decline in the numbers of SE cells in albumen has been noted during refrigerated storage (Stephenson *et al.*, 1991). After inoculation into the albumen of whole eggs, at sites remote from the yolk,

Table 1.1 Principal antibacterial proteins in albumen of chicken eggs

Protein	Proportion of total protein (%)	Antibacterial properties
Ovotransferrin	12	Binds iron and other metal ions
Ovomucoid	11	Inhibits activity of trypsin
Lysozyme	3.4	Causes lysis by hydrolyzing β-1,4 glycosidic bonds in cell walls
Ovoinhibitor	1.5	Inhibits several proteases
Ovoflavoprotein	0.8	Binds riboflavin
Avidin	0.06	Binds biotin

Sources: Derived from information in Burley and Vadehra (1989), Board and Fuller (1994), and Stadelman and Cotterill (1995).

Table 1.2 Changes in the contents of chicken eggs during storage

Component	In eggs at oviposition	In eggs after storage
Air cell	Small	Larger, due to loss of water and CO_2 from albumen
Albumen	Firm, holds yolk in center; pH ~ 7.6	Thinner, more fluid and less gelatinous, because of water and CO_2 loss; pH ~ 9.5
Yolk	Denser than albumen	Enlarged and less dense, due to water uptake from albumen following degradation of vitelline membrane integrity

Sources: Adapted from information in Burley and Vadehra (1989), Board and Fuller (1994), and Stadelman and Cotterill (1995).

a modest degree of growth has sometimes been observed after several days of incubation at 20 ºC or higher (Gast and Holt, 2000b; Cogan *et al.*, 2001). Multiplication of SE proceeds faster in fresh than in stored albumen, possibly due to an increase in pH during storage (Messens *et al.*, 2004). Perhaps by inactivating ovotransferrin and other antibacterial proteins, pasteurization has been found to render albumen less resistant to bacterial growth (Baron *et al.*, 1999).

In egg yolk, nutrients are present in abundance and the antimicrobial albumen proteins are absent, so the growth of SE can be rapid and prolific (Clay and Board, 1991). Even very small initial numbers of SE cells can multiply to reach dangerously high concentrations within a single day, after inoculation into egg yolk (Gast and Holt, 2000b). Temperature is the principal factor that affects SE growth in egg yolks. Extensive multiplication has been reported at 15 ºC and higher, whereas slower multiplication is evident at 10 ºC and growth ceases at around 4 ºC (Kim *et al.*, 1989; Saeed and Koons, 1993; Schoeni *et al.*, 1995; Gast and Holt, 2000b).

Even if SE is not located initially inside the yolk contents of contaminated eggs, but, instead, is deposited on the exterior surface of the vitelline membrane or in nearby areas of the albumen, bacterial penetration through the membrane could still result in extensive multiplication within yolks. Using various *in vitro* models for egg contamination, the penetration of SE through the yolk membrane has been reported to occur at a wide range of frequencies (Hammack *et al.*, 1993; Humphrey and Whitehead, 1993; Braun and Fehlhaber, 1995; Gast and Holt, 2000b). However, in a similar study, no movement of *Salmonella* from the exterior to the interior of the yolk membrane was observed (Fleischman *et al.*, 2003). The migration of SE across the vitelline membrane into the yolk has been shown to increase with the level of contamination, storage temperature and egg age (Braun and Fehlhaber, 1995; Gast and Holt, 2000b).

Another mechanism by which SE could eventually begin to multiply rapidly after deposition in the albumen involves the gradual degradation of the vitelline membrane, leading to the release of yolk nutrients into the albumen, as the egg

ages (Humphrey, 1994; see also Table 1.2). This deterioration of the yolk membrane is accelerated by abusively high temperature conditions (Hara-Kudo *et al.*, 2001; Latimer *et al.*, 2002). In experimentally contaminated eggs, the growth of SE in areas of the albumen around the yolk increased with the age of the eggs at inoculation (Humphrey and Whitehead, 1993). However, rapid growth of SE in albumen, due to yolk-membrane degradation, has been observed after only three weeks of storage at 20 ºC (Humphrey and Whitehead, 1993).

1.3.3 Implications for detecting *Salmonella* Enteritidis in eggs

The nature of SE deposition in eggs has a profound effect on the methods that have evolved for detecting contamination. Because SE deposition is evidently a highly infrequent event and, because contaminated eggs have usually been found to contain very low concentrations of SE cells, large numbers of eggs must be sampled to ensure that the pathogen is detected with adequate sensitivity (Gast, 1993). This imposes several significant constraints on practical and dependable methods for detecting the organism in eggs taken from commercial laying flocks. To sample large numbers of eggs without overwhelming available laboratory resources, the contents of up to 20 eggs are often pooled together. However, pooling eggs introduces a dilution of the already small numbers of SE cells. Accordingly, incubation of egg pools, before applying subsequent enrichment culture steps, is essential to permit the multiplication of SE to more consistently detectable levels (Gast, 1993; Gast and Holt, 2003). Supplementing these pools with concentrated sources of iron and other nutrients can improve the growth rate of SE in incubating egg content pools (Gast and Holt, 1998b; Chen *et al.*, 2001). Innovative rapid technologies for detecting SE can be applied to eggs to replace traditional culture methods, but are still dependent on a preliminary egg-pool incubation step to achieve satisfactory detection sensitivity (Gast and Holt, 2003).

1.3.4 Implications for refrigeration or pasteurization of eggs

The nature of SE deposition in eggs also has significant consequences for the application of refrigeration or pasteurization as measures to protect consumers from egg-transmitted illness. Refrigeration of eggs at 7 ºC during storage and transportation has been recommended repeatedly for preventing the multiplication of small initial numbers of SE cells to more dangerous levels (US Department of Agriculture, 1998; US Food and Drug Administration, 2004). However, refrigeration of eggs using conventional technologies may require several days before temperatures within the eggs are reduced sufficiently to prevent further microbial growth (Curtis *et al.*, 1995; Thompson *et al.*, 2000). If SE is deposited in the albumen, where bacterial multiplication is very slow, even at warm temperatures, the extended interval necessary to achieve growth-restricting temperatures inside eggs will have little adverse effect. However, if the initial site of SE contamination is in or on the nutrient-rich yolk, rapid

multiplication could produce high levels of the pathogen during the early stages of refrigerated storage, while internal egg temperatures are still in the process of being reduced. Determining how often SE is in fact deposited in association with the yolk, and whether (and how quickly) it can penetrate through the vitelline membrane into the yolk, is, accordingly, very important in defining the necessary parameters for thoroughly protective application of egg refrigeration. Many of these same considerations affect the ultimate efficacy of egg pasteurization, as the number of bacteria that will survive destruction by heat (either inside intact shell eggs or in liquid egg products) under any specific combination of time and temperature will depend on the numbers of cells that were present initially (Hou *et al.*, 1996; Brackett *et al.*, 2001). Therefore, the effect of the location of deposition on subsequent growth to high numbers before pasteurization becomes a pivotal consideration in this context as well. Techniques for reducing pathogens in eggs are discussed in more detail in Chapter 12.

1.4 Future trends

Considerable public and private resources have been invested throughout the world in attempting to control the egg-borne transmission of SE. A risk assessment study performed in the USA recommended intervention at multiple steps in the farm-to-fork continuum, as the most productive overall strategy (Hope *et al.*, 2002). As already discussed in the previous section, refrigerated storage and pasteurization of eggs are highly effective post-production options for risk mitigation. Nevertheless, the preponderance of effort and expenditure has been devoted to controlling SE infections in laying flocks. In the early years after SE was first identified as a significant public health problem, most control plans focused on trace-back testing and eradication efforts. For example, in a national programme that was instituted in the USA from 1990 to 1995, flocks were tested after being implicated as the sources of eggs that had caused human disease outbreaks. This plan mandated either the diversion of eggs for pasteurization or depopulation of the laying house, when the flock was found to be infected (US Department of Agriculture, 1991; Hogue *et al.*, 1997b). During the term of this control programme, restrictions were imposed on 31 laying flocks, resulting in the voluntary depopulation of nearly nine million laying hens and the diversion of more than one billion eggs for pasteurization. However, during this same period of time, the overall incidence of SE in both poultry and eggs in the USA continued to increase (Hogue *et al.*, 1997a). The apparent failure of this entirely reactive trace-back approach illustrates the inherent impossibility of identifying and eradicating all infected flocks in the face of continuous re-introduction of SE into laying flocks from diverse environmental sources.

In recent years, an assortment of microbial quality-assurance programmes for commercial laying flocks have been proposed and implemented by government

agencies and by the poultry industry (Hogue *et al.*, 1997b; US Food and Drug Administration, 2004). These programmes have represented a more proactive, and thereby far more effective, alternative to trace-back eradication. Most of these programmes combine a battery of risk-reduction practices for egg producers, with a testing component designed to identify problem flocks for further attention (sometimes including regulatory intervention). The testing part of these programmes also serves as a means of assessing the ongoing efficacy of the risk-reduction practices to ensure that the commitment of resources to quality assurance programmes is cost-effective. In the most common approach to testing, environmental samples are collected and tested to screen for flock infection, and egg samples are subsequently tested to determine whether an ongoing threat to public health exists. Eggs from flocks that test positive must generally be diverted for pasteurization (Hogue *et al.*, 1997b; US Food and Drug Administration, 2004). Risk-reduction practices that are common to most quality assurance schemes include using chicks from flocks that are certified as uninfected by breeder-flock testing protocols, such as those of the National Poultry Improvement Plan in the USA (Rhorer, 1999), implementing effective procedures for controlling rodents and other pests, heightened biosecurity measures for poultry facilities, thorough cleaning and disinfection of facilities between flocks and refrigeration of eggs as soon as possible after collection. This type of approach has been associated with significant reductions in the incidence of SE infections in both egg-laying flocks and humans in several states in the USA (White *et al.*, 1997; Mumma *et al.*, 2004).

Another important tool for combatting SE infection in poultry is vaccination. Vaccination of pullets or hens with either killed or live preparations has reduced (but not entirely prevented) faecal shedding, organ invasion and egg contamination, following challenge with SE (Gast *et al.*, 1992, 1993; Zhang-Barber *et al.*, 1999). This protection can be particularly significant for highly susceptible hens undergoing an induced molt (Holt *et al.*, 2003; Nakamura *et al.*, 2004). However, vaccination does not construct an impenetrable barrier to SE infection, since protective immunity induced by vaccines has been overcome occasionally by high challenge doses. A field study in the USA found no significant protective effect against SE that could be attributed to vaccination of commercial laying flocks (Davison *et al.*, 1999). Poor vaccine performance has sometimes been tied to severe rodent or sanitation problems in laying houses (Davies and Breslin, 2003b). Nevertheless, even when vaccination has not completely prevented SE infection in commercial flocks, it has generally been able to accomplish meaningful reductions in egg contamination (Davies and Breslin, 2004). In the UK, a declining prevalence of SE infections in humans was observed to follow the initiation of widespread vaccination of laying hens (Cogan and Humphrey, 2003). Vaccination may be most valuable as an adjunct to other risk-reduction practices, especially when applied to highly susceptible flocks or flocks exposed to severe challenges from environmental sources.

The most promising option for achieving sustainable reductions in the prevalence of contaminated eggs appears to be the patient and persistent

application of risk-reduction programmes of verified efficacy. However, one potential area of vulnerability in microbial quality-assurance schemes for shell eggs is created by the possibility that *Salmonella* serotypes other than SE might become significant sources of egg-transmitted human disease. Although the epidemiological association between SE and eggs has been strong and unique, other paratyphoid serotypes (including *S.* Typhimurium, *S.* Heidelberg and *S.* Thompson) have also been reported to be capable of colonizing reproductive organs of chickens and thereby causing egg contamination (Snoeyenbos *et al.*, 1969; Cox *et al.*, 1973; Keller *et al.*, 1997; Okamura *et al.*, 2001b; Gast *et al.*, 2004). Recently, the Centers for Disease Control and Prevention in the USA have implicated eggs and egg-containing foods as the principal sources of human *S.* Heidelberg infections (Hennessy *et al.*, 2004). Nevertheless, several pivotal aspects of current risk-reduction efforts, such as testing and vaccination, focus almost exclusively on identifying or controlling SE and are not intended to address the possible presence of other *Salmonella* serotypes in eggs. Although targeting control measures to specific disease agents is crucial for mounting rapid responses to public health emergencies, risk-reduction practices that are not inherently agent-specific (such as biosecurity, rodent control, cleaning and disinfection and egg refrigeration) may be of even greater long-term importance because of their ability to minimize the opportunities for another pathogen to emerge and cause a new egg-borne disease crisis.

1.5 Sources of further information and advice

The most comprehensive, single source of information about SE in eggs and chickens is Saeed *et al.* (1999). This book contains 39 chapters relating to the subject, subdivided into sections on international public health issues, molecular epidemiology, virulence and pathogenesis, and prevention and control. General texts that provide extensive background information about eggs (including microbiological considerations) are Burley and Vadehra (1989), Board and Fuller (1994) and Stadelman and Cotterill (1995). Despite having been written ten years ago, a review of egg contamination problems by Humphrey (1994) remains very useful in its treatment of the principal issues. The most thorough description of avian *Salmonella* infections is found in Gast (2003). Although it covers other domestic animals in addition to poultry, several chapters in Wray and Wray (2000) provide good coverage of central themes relating to *Salmonella* in chickens. Guard-Petter (2001) offers a thought-provoking review of the mechanisms by which SE causes egg contamination. The epidemiology of human SE infections in the USA is addressed by Hogue *et al.* (1997b) and Patrick *et al.* (2004). The government-sponsored risk assessment for SE in eggs in the USA is described by Hope *et al.* (2002). The record of the effectiveness of egg-quality assurance programmes in influencing the epidemiology of SE in the USA is documented by Mumma *et al.* (2004). Considerable information about both the SE problem and responses to it by public health and regulatory agencies

can be found in the corresponding official websites (examples are www.cdc.gov, www.fda.gov, and www.fsis.usda.gov in the USA). A particularly good presentation of a state egg-quality assurance scheme is found at http://ulisse.cas.psu.edu/ext/Comeggs.html.

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2

Bacterial contamination of poultry as a risk to human health

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2.1 Introduction

Bacteria present on poultry meat can be divided into two groups:

(1) pathogenic (those capable of producing disease in humans) and
(2) non-pathogenic (those not previously associated with a recognized disease).

Many of the organisms in the non-pathogenic group are important because of their spoilage capabilities. In this chapter, the following foodborne pathogens will be discussed in detail: *Campylobacter* spp., *Clostridium perfringens, Listeria monocytogenes, Salmonella enterica* subsp. *enterica, Staphylococcus aureus*, pathogenic *Escherichia coli* and *Yersinia enterocolitica*. Also considered are factors affecting symptomless carriage of pathogens in poultry, routes of transmission into the supply chain and possible control measures.

2.2 Foodborne human pathogens associated with poultry

2.2.1 *Campylobacter* spp.

The term 'campylobacter' was proposed by Sebald and Veron (1963) as the generic name for microaerophilic vibrios, because these organisms differed from the classical cholera and halophilic groups. It was suggested that *V. fetus* should be removed from the genus *Vibrio* and be re-classified as *Campylobacter fetus*, the type species of the genus.

Campylobacters are Gram-negative, slender, curved or spiral-shaped rods (Corry *et al.*, 1995). They are motile by means of a single polar flagellum, although occasionally they have a polar flagellum at each end of the cell. For the most part, campylobacters require a microaerobic atmosphere for growth and can be very difficult to work with in laboratory settings, due to their fragile nature.

C. jejuni is the species of greatest concern to the food microbiologist. There are many reservoirs of *C. jejuni* in nature. The organism has been isolated from cattle, sheep, swine, poultry, dogs, cats and monkeys, and also from fresh water and sea water (Karmali and Fleming, 1979; Stern, 1992). *Campylobacter* is among the leading causes of acute bacterial gastroenteritis in humans (Slutsker *et al.*, 1998). Contaminated food and water are often implicated as the main vehicles responsible for transmitting *C. jejuni* to susceptible individuals (Stern, 1992; Berrang *et al.*, 2003). Foods of particular concern in this respect are raw milk, beef and poultry (Stern, 1992). Commercial poultry are known to be a major reservoir of *C. jejuni* (Bryan and Doyle, 1995; Cox *et al.*, 2002a,b; Berrang *et al.*, 2003; Herman *et al.*, 2003); however, the precise extent to which poultry consumption or handling is a cause of human campylobacteriosis is still a matter of debate.

The illness has been shown to follow cross-contamination of foods from the mishandling of raw poultry or consumption of undercooked poultry meat (Park *et al.*, 1981; Kinde *et al*., 1983; Bryan and Doyle, 1995). Poultry and poultry-related products are common niches for *Campylobacter* spp. and the organisms seem to survive well in these environments, contrary to their behavior in a laboratory setting. Variation exists in the reported incidence and number of *Campylobacter* spp. on raw poultry, and this is due primarily to past inadequacies in recovery techniques for the organisms (Waldroup, 1996). Recovery techniques have improved dramatically over the last decade, but more sensitive techniques could prove to be beneficial to determine more accurately the level of *Campylobacter* contamination of poultry and routes of transmission of the organisms (Corry *et al.*, 1995, Cox *et al.*, 2002a).

A related organism, *Arcobacter*, is more aerotolerant than *Campylobacter*. This organism has been isolated from water, sewage, poultry and pork (Lammerding *et al.*, 1996; Rice *et al.*, 1999). Frequently isolated from poultry skin and meat, arcobacters are seldom recovered from the avian intestinal tract (Wesley and Baetz, 1999). Rivas *et al.* (2004) detected *A. butzleri* in poultry (73%), pork (29%), beef (22%), and lamb (15%). Collins *et al.* (1996) recovered *Arcobacter* spp. from approximately 90% of pork samples. Houf *et al.* (2002) found *Arcobacter* more often than *Campylobacter* on poultry carcasses (96% v. 49%). These researchers also isolated the organism from the slaughterhouse environment and from processing equipment. *A. butzleri* and *A. cryaerophilus* have been associated with human enteritis, but risk factors have not been established. *Arcobacter* has been identified as the etiological agent of diarrhea and bacteremia in newborn infants, young children, elderly people and patients with a pre-disposing illness (Vandamme *et al.*, 1992; On *et al.*, 1995; Lauwers *et al.*, 1996; Marinescu *et al.*, 1996; Hsueh *et al.*, 1997).

2.2.2 *Clostridium perfringens*

Cl. perfringens is an anaerobic, Gram-positive, spore-forming, rod. The organism is differentiated into five types (A–E) on the basis of certain major lethal toxins. In addition, types A and C are able to produce an enterotoxin associated with human gastroenteritis, but type A is the more common cause of foodborne illness (Walker, 1975).

The organism is commonly found in the environment and is a frequent inhabitant of the intestinal tract of both humans and poultry. Poultry carcasses can become contaminated from soil, feces or hands of operatives during slaughtering and processing. Mead and Impey (1970) studied the distribution of clostridia in a number of chicken and turkey plants and found that the organisms could be isolated readily from carcasses at each stage of processing. Of the *Clostridium* spp. isolated in chicken plants, 91% were *Cl. perfringens*, compared with 56% in the turkey plants. Birds entering the processing plant carry a relatively large number of *Cl. perfringens* among the breast feathers and on the skin and feet. After scalding and plucking, very few remain on the carcass and they can only be recovered in significant numbers from the cecum (Barnes, 1960).

Most organisms that could compete with vegetative cells of *Cl. perfringens* are killed when meat and poultry are cooked, but *Cl. perfringens* spores can survive. In many cases, the spores are unusually heat-resistant and may be heat-shocked during cooking, so that more of them germinate as cooked meats and gravies cool, while being held at room temperature, in food-warming devices or even in refrigerators. Also, workers can contaminate cooked meat during boning, slicing, grinding, mixing or handling, if any of their equipment is contaminated with *Cl. perfringens*. When foods are stored in large containers, the spores may germinate and vegetative cells multiply to levels that can cause illness, unless the foods are subsequently reheated to temperatures that are sufficient to kill the vegetative form of the organism. Therefore, when meat- or poultry-borne outbreaks occur, one or more of the following events has usually been responsible:

- improper cooling
- improper hot-holding
- food prepared a day or more before serving and stored badly
- inadequate reheating (Bryan, 1978, 1980).

2.2.3 *Listeria monocytogenes*

Following the 1985 listeriosis outbreak in California, USA, public concerns were raised about the safety of dairy products and other potentially contaminated foods, including meat and poultry products. The concern about the safety of raw poultry soon escalated after a nationwide telecast informed the public that about 50% of all raw chicken marketed in the USA was contaminated with *Salmonella*.

These reactions prompted officials in the US Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) to initiate a poultry testing program in January 1989 for *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7. As a result, *L. monocytogenes* and *Salmonella* were detected in 18.0% of 2686 samples and 28.9% of 2739 samples respectively, with *E. coli* O157:H7 being absent from 2696 samples (Anon, 1990). Bailey *et al.* (1989) determined the incidence of *L. monocytogenes* and other *Listeria* spp. on broiler carcasses processed in the southeastern United States. The fact that most *L. monocytogenes* strains isolated from chickens were pathogenic to mice suggested chicken meat as a possible vehicle in human cases of listeriosis.

In one survey, *L. monocytogenes* was isolated from 59% of raw samples, but all cooked samples were negative for the organism (Lawrence and Gilmour, 1994). Ryser *et al.* (1996) found *Listeria* spp. in 34 of 45 retail samples of chicken portions, with 11 different ribotypes of *L. monocytogenes*, including three associated with previous cases of foodborne listeriosis, being detected among the *Listeria*-positive samples. These findings suggest the presence of multiple sources of contamination and/or heavily-contaminated, single sites within the poultry processing environment. Hence, improved disinfection procedures are likely to be necessary for any effective control of *Listeria* spp. in processing.

In a study by Cox *et al.* (1997), *L. monocytogenes* was occasionally found in hatchery samples and on the exterior of fully-grown birds. Although the organism was not recovered from the intestinal tract of broiler chickens at the time of slaughter, 25% of post-processing, retail carcasses contained *L. monocytogenes.* In another study, Kwantes and Isaac (1975) detected *L. monocytogenes* on the internal and/or external surfaces of 27 of 51 (52.9%) raw chickens obtained from a local processor, with 23 (85.2%) and 4 (14.8%) isolates, respectively, being identified as serotype 1 and 4b. To determine the extent of consumer exposure to contaminated poultry, these investigators went to the homes of poultry purchasers in Wales, UK, and swab-sampled locally-purchased carcasses of fresh and frozen chicken, turkey, duck and pheasant. *L. monocytogenes* was isolated from 50% of fresh chicken taken from home refrigerators and from 64% of frozen chickens stored in home freezers, demonstrating the ability of this pathogen to persist on frozen carcasses. The carcasses most likely become contaminated with *Listeria* during evisceration and subsequent handling in the processing plant, as suggested by Genigeorgis *et al.* (1989, 1990) and Cox *et al.* (1997) in surveys of processing facilities in California and Georgia, USA.

The Centers for Disease Control and Prevention estimate about 2500 cases and 500 deaths per year in the USA from *L.* monocytogenes (Mead *et al.*, 1999). The proportion of these cases that comes from poultry products is difficult to determine. There is evidence that poultry and other deli meats have contributed to the illnesses and deaths that have occurred. One outbreak originating from a deli meat-producer in the winter of 1998–99 resulted in 15 deaths and 101 people becoming sick, with a resultant recall of 35 million pounds of meat.

Raw-meat products containing *Listeria* spp. can be sold in most countries of the world, including the USA, and there is no evidence that these present any significant, direct risk to human health. Canada, Germany and some other countries have a tolerance limit of 100 cells of *Listeria* per gram for products with secondary growth-barriers, such as high acidity or low water activity, and a similar situation exists for cooked, ready-to-eat poultry in the UK. The USA, on the other hand, has a zero tolerance for *Listeria* on all ready-to-eat food products, whether or not they possess secondary growth barriers. While there is evidence that *Listeria* is present at low levels in a wide variety of food products and relatively few people become sick as a result, the occurrence of specific outbreaks related to deli meat products makes it difficult for the US regulatory authorities to change their policy. In an effort to gain a better understanding of these issues, improved risk assessment models are currently being developed.

2.2.4 *Salmonella* serotypes

Raw foods, particularly those of animal origin, have long been known as major vehicles of foodborne salmonellosis. Poultry, beef and pork are the most important sources of human salmonellosis (Mishu *et al.*, 1994; Mossel, 1988; Olsen *et al*., 2000). The estimated annual incidence of foodborne illness from *Salmonella* in the USA ranges from one to four million cases (Tauxe, 1996; Mead *et al.*, 1999).

Although commercial feed examined in the past was often contaminated with *Salmonella*, surveys showed that the proportion of birds arriving at the processing plant with salmonellas in their intestinal tracts was low, approximately 2% for chickens and 2–5% for turkeys (Sadler *et al.*, 1961; Sadler and Corstvet, 1965). Once inside the processing plant, however, there would be widespread dissemination of microorganisms, including salmonellas, during processing, leading to contamination of the final product. May (1974) listed 57 potential points in a poultry processing plant at which cross-contamination could occur. In consequence, there would be a higher incidence of contaminated carcasses leaving the processing plant and passing on to the retail market.

There is much published information on the incidence of *Salmonella*-contaminated poultry. Earlier studies carried out throughout the world were reviewed by Waldroup (1996). While contamination rates varied considerably between studies, it is evident that *Salmonella* contamination of poultry products was common and frequently between 20% and 50%. In other work, Glezen (1966) reported 33% positive from one plant and only 0.5% from another; Wilder and MacCready (1966) found 48.7% and 28.8% respectively at two different processing plants, while Cox and Blankenship (1975) reported 80, 65, 35 and 5% respectively at four separate processing plants. Johnston (1982) presented data from paired surveys carried out in 1967 and 1979, using the same methods of sampling and testing and most of the same 15 federally-inspected processing plants. From approximately 600 samples taken in each study, the incidence of contaminated carcasses was 28.6% in 1967 and 36.9% in 1979.

More recent scientific data indicate that the situation has not changed significantly compared to past years. For example, contamination rates of 4.2% to 53% have been reported (Dufrenne *et al.*, 2001; Harrison *et al*., 2001; Zhao *et al*., 2001; Simmons *et al*., 2003). On the other hand, a large survey of retail poultry in the UK (Report, 2003) revealed that only 5.7% were *Salmonella*-positive, the lowest figure for many years (Report, 1996). Although the proportion of contaminated carcasses is frequently high, levels of the pathogen on positive carcasses tend to be rather low, e.g. 1–30 cells per carcass (Surkiewicz *et al.*, 1969, Campbell *et al*., 1983, Waldroup *et al.*, 1992) or 100 cells per 100 g of skin (Mulder *et al.*, 1978). Such low levels are below those normally associated with human salmonellosis and suggest that considerable multiplication must occur as a result of mishandling the meat to cause illness.

In developed countries around the world, *Salmonella* Enteritidis emerged during the late 1980s and early 1990s as the most frequent cause of salmonellosis from foods prepared with raw or undercooked eggs (Poppe, 1999). In the UK, producing eggs under British Lion Quality standards, which include vaccination of hens against *S.* Enteritidis, is said to have been responsible for a 54% decrease in egg-borne salmonellosis (British Egg Information Service, 2004). In the USA, attention to farm biosecurity, rodent control and farm hygiene, as well as maintaining proper washing conditions and refrigeration of eggs from processing through the retail chain, have all helped to reduce the prevalence of *S*. Enteritidis (United Egg Producers, 2004). Egg-borne salmonellosis caused by *S*. Enteritidis has decreased significantly in the USA (Marcus *et al*., 2004); however, other serotypes, such as *S*. Heidelberg, have been associated with egg-borne disease, particularly when eggs are consumed outside the home (Hennessy *et al*., 2004).

In surveys of commercial plants, 5–10% of eggs were contaminated with *Salmonella* (Jones *et al.*, 1995; Musgrove, 2004). Shell eggs are washed in Canada, Japan and the US. Wash-water temperature and pH, fresh-water quality and good plant hygiene increase the efficacy of washing as a means of reducing *Salmonella* contamination of shell eggs (Musgrove, 2004).

Poultry is a major source of *Salmonella* and it is obvious that at least small numbers of these organisms enter the kitchens of many homes, institutions or food-service establishments at one time or another. Bryan (1978) lists various factors that have contributed to outbreaks of salmonellosis and some of the more important ones are:

- inadequate cooling of cooked foods
- ingestion of contaminated raw foods or ingredients
- inadequate time and/or temperature during cooking or heat processing
- cross-contamination from raw foods (such as raw poultry and red meat) to cooked foods
- a lapse of a day or more between preparing and serving the food
- inadequate cleaning of equipment and kitchen surfaces.

From the number of reported outbreaks and cases involving *Salmonella*, it is

obvious that salmonellosis is an important public health problem, both in the USA and in other developed countries worldwide.

2.2.5 *Staphylococcus aureus*

Strains of *Staph. aureus* are frequently carried by live chickens or turkeys. The organism enters the processing plant on the skin or in the nasal cavity of many birds and can be found subsequently in low numbers throughout the plant (Mead, 1989; ICMSF, 1998). Also, the organism can be associated with bruised tissues (Bryan, 1976). If poultry meat that is intended for further processing shows unusually high numbers of *Staph. aureus*, it may be considered unsuitable for the purpose (Mead and Dodd, 1990). For example, some batches of mechanically deboned meat with a high skin content can have increased levels of staphylococci (Waldroup, 1996; Holder *et al.*, 1997). Numbers of *Staph. aureus* are generally lower in samples taken later in the operating day at broiler slaughter plants (Whyte *et al*., 2004).

The degree of colonization and the numbers of *Staph. aureus* present are low during the first few weeks of a chick's life, but tend to increase as the chick grows older (Thompson *et al*., 1980). Patterson (1969) found that broiler carcasses normally yield fewer than 50–100 cells of *Staph. aureus* per 16 cm^2 of breast skin, although counts as high as 750 cells per cm^2 were reported. Waldroup (1996) listed reports on the incidence and numbers of *Staph. aureus* isolated from poultry products worldwide, showing that high numbers (more than 10^3 cfu/cm^2) were found in some studies. Notermans *et al.* (1982) found that *Staph. aureus* was present in small numbers (about 10 cells/g) on the skin of live broilers, but, during processing, contamination of carcasses with this organism increased to more than 10^3 cfu/g of skin. Counts of *Staph. aureus* from older hens and turkeys were shown to be higher (more than 10^3/cm^2) than on broiler carcasses (Gibbs *et al*., 1978; Thompson *et al*., 1980). The recent chicken and turkey baseline studies in the USA (USDA-FSIS, 1996, 1998) found *Staph. aureus* on about 65% of immersion-chilled carcasses, with mean levels of less than 10 cfu/cm^2, when carcasses were sampled by rinsing.

Surkiewicz *et al.* (1969) found *Staph. aureus* in low numbers on eviscerated broiler carcasses and in chill-tank water, but showed that the washing action of continuous mechanical chillers reduced the numbers on carcasses still further. In another study, da Silva (1967) sampled meat, equipment and personnel in turkey processing plants that produced rolls and roasts. Coagulase-positive staphylococci were isolated from several locations in the plants and from uncooked, final products, but not from cooked rolls. Hands of workers, defeathering operations and chill-tank water contributed to increases in the incidence of *Staph. aureus* on raw poultry meat. Notermans *et al.* (1982) found plucking and evisceration to be the main processing steps at which contamination of carcasses with *Staph. aureus* occurred.

Many strains of *Staph. aureus* on the skin of the live bird are removed or killed during carcass scalding, but the carcasses can be recontaminated during

defeathering (Mead and Dodd, 1990; ICMSF, 1998). Staphylococci are endemic in the defeathering equipment of some plants and this may add to the levels of carcass contamination. *Staph. aureus* is somewhat unusual among foodborne-disease agents isolated from processed poultry, in that the bacterium can also cause significant disease problems in chickens and turkeys, although the strains involved are usually different (Capita *et al.*, 2002). Staphylococci on processed carcasses should be destroyed by proper cooking and staphylococcal foodborne illness in humans is usually due to recontamination of the cooked meat by an infected food handler (Cox and Bailey, 1987; ICMSF, 1998). If the contaminated food is not refrigerated properly, *Staph. aureus* can multiply and produce enterotoxin. Meat and poultry products were implicated in 75% of staphylococcal food poisoning incidents in the UK between 1969 and 1990 (Wieneke *et al.*, 1993). Hobbs (1971) suggested that poultry are likely to acquire their staphylococci from human sources, but subsequent research, using phage typing, has shown that both strains specific to humans and others indigenous to poultry can be isolated. Most poultry strains do not produce the enterotoxins that cause human foodborne disease (Isigidi *et al.*, 1992; ICMSF, 1998; Hazariwala *et al.*, 2002).

Methicillin-resistant *Staph. aureus*, similar to strains causing disease in humans, has been isolated from chickens in Korea (Lee, 2003). However, transfer of antibiotic resistance between strains from animals and humans is thought to be infrequent (Kaszanyitzky *et al.*, 2003).

2.2.6 Pathogenic *Escherichia coli*

Verocytotoxin-producing strains of *E. coli* (VTEC) cause diarrhea and hemorrhagic colitis in humans and are sometimes associated with potentially life-threatening sequelae, such as hemolytic uremic syndrome. Although VTEC strains occur in a wide range of O-serogroups, the most important in human disease is O157, which accounts for almost all major foodborne outbreaks in Europe and the USA (Report, 1996). The organism was first recognized as a pathogen in 1982, when outbreaks occurred in the USA from consumption of contaminated hamburgers (Riley *et al.*, 1982). While VTEC O157 is mostly found in ruminant animals, it is occasionally associated with other livestock and various foods of animal origin, including poultry. In a survey of retail meats in the USA, Doyle and Schoeni (1987) found VTEC O157 in 1.5% of 263 samples of chicken and turkey leg meat. Although Heuvelink *et al.* (1999b) could find no VTEC O157 in the feces of Dutch chickens, 1.3% of 459 pooled samples from turkeys were positive and one isolate contained genes for type 2 verotoxin, attaching-and-effacing capability and the relevant hemolysin. Because of these virulence factors, the strain appeared capable of causing illness in humans. Only turkeys had been kept on the farm in question, so transfer of the strain from other livestock was unlikely in this case. VTEC other than O157 were found in 12% of retail chicken samples and 7% of turkey samples in the USA by Samadpour *et al.* (1994), but their pathogenic potential was not determined further.

Surveys by the Food Safety Inspection Service of the United States Department of Agriculture found no *E. coli* O157 in 1297 chicken carcasses (USDA-FSIS, 1996) or 1221 turkeys (USDA-FSIS, 1998) sampled immediately after immersion chilling. In the United Kingdom, Chapman *et al.* (1997) found no O157 in 1000 fecal samples taken immediately after slaughter. Caya *et al.* (1999) compared *E. coli* isolates from healthy and infected chickens with isolates from sick humans in the same geographic area and found no O157 in the chickens and no relationship between avian and human isolates. Heuvelink *et al.* (1999a) tested 819 poultry meat samples from retail shops in the Netherlands and found no O157. Similarly, samples of 274 chicken breasts and carcasses in Belgium (Tutenel *et al.*, 2003) and 216 carcasses in the Czech Republic (Lukasova *et al.*, 2004) were negative for *E. coli* O157.

Only rarely are poultry meat products incriminated in VTEC outbreaks. An outbreak in the United Kingdom that was associated with eating turkey roll was reported by Salmon *et al.* (1989) and two further outbreaks linked to chicken dishes were recognized by Kessel *et al.* (2001). In all three outbreaks, the causative organism was *E. coli* 0157:H7; however, cross-contamination in the kitchen was suspected by the public health authorities (Gillespie, 2003). In a case-control study of a hemolytic-uremic syndrome outbreak in Italy (Tozzi *et al.*, 1994), the only significant association was exposure to chicken coops (odds ratio = 6.5), although VTEC were not isolated from the coops themselves.

Wild birds are a potential source of infection for poultry. VTEC O157 was found in samples of feces from wild gulls at landfills (0.1%) and inter-tidal sediments (2.9%) in the UK (Wallace *et al.*, 1997). In Finland, Shiga toxin-producing *E. coli* from gulls, pigeons and broiler chickens were found to be different from strains known to be pathogenic for humans (Kobayashi *et al.,* 2002). The bird isolates lacked key virulence factors and showed a different O-serogroup distribution than human strains.

Despite the relative rarity of VTEC O157 in poultry, experimental studies have shown that chicks can be colonized readily with a challenge dose as low as 10 cfu/bird (Schoeni and Doyle, 1994) and colonization may persist for at least three months. Another study (Stavric *et al.*, 1993) showed that the organism was present, following challenge, on cecal mucosa and in the contents of the lumen. The extent of colonization depended on dose, age, breed and time after exposure. *E. coli* O157 was reported in a few poultry samples in Canada (Onderka *et al.*, 1997) and France (Vernozy-Rozand *et al.*, 1997), but isolates in both studies were negative for the H7 antigen and did not produce toxin. Isolates of *E. coli* O157 from Libyan poultry (Pilipcinec *et al.*, 1999) were also negative for the H7 antigen. Sampling of German turkey flocks found VTEC O157 in two of eleven flocks during rearing and VTEC were isolated from all flocks during processing, although not all isolates were O157 (Hafez *et al.*, 2001). *E. coli* O157 has also been isolated from mechanically deboned poultry meat in the Czech Republic, including some H7.

Since VTEC O157 is capable of colonizing poultry without causing illness in the birds, is present in other livestock and survives well in soil (Reissbrodt *et al.*,

1999), it is surprising that the organism is not found more often in commercial broiler flocks. Further information is needed on the behavior of this organism and other VTEC strains in the poultry-production environment.

2.2.7 *Yersinia enterocolitica*

From the food microbiologist's point of view, *Yersinia enterocolitica* is of concern, because it is one of the few human pathogens that can grow well in refrigerated foods (0–5 ºC). The psychrotrophic nature of this organism presents a particular problem to the food industry in relation to food safety. *Y. enterocolitica* has been isolated from a variety of animals and, among the most common, are swine, which harbor one of the serotypes (0:3) that are usually associated with human disease in Europe and Japan. For this reason, swine can be considered a significant source of yersiniosis (Toma and Diedrick, 1975; Schiemann, 1980).

Leistner *et al.* (1975) isolated *Y. enterocolitica* from 29% of 121 samples of chicken meat. In examining 82 samples of frozen chicken obtained from retail stores in Sweden, Norberg (1981) recovered *Y. enterocolitica*, *Campylobacter* and *Salmonella* from 20, 18 and 1 of the samples, respectively. De Boer *et al.* (1982) analyzed 108 poultry samples for the presence of *Y. enterocolitica*. They sampled raw chicken carcasses, parts such as livers, gizzards, legs, wings, necks and breasts, as well as further-processed products, such as sausages, rolled roasts and 'chicken burgers'. Of the 108 samples tested, 73 (68%) were positive for *Y. enterocolitica.* For 14 of the samples, they enumerated the *Y. enterocolitica* present, using a three-tube Most Probable Number procedure, and found that numbers varied from 30 to 1.1×10^4 per gram, with a mean of 1.8×10^3 per gram. Cox and Bailey (1987) recovered *Yersinia* from 34 of 60 (57%) broiler carcasses sampled. More than one species of *Yersinia* were isolated from 11 carcasses and 9 of 60 (15%) were contaminated with *Y. enterocolitica.* Clearly the latter organism is common on poultry carcasses, but the serotypes that are pathogenic for humans are not usually found. Although yersiniosis is frequently suspected as an agent of foodborne disease, the linkage between *Y. enterocolitica* in foods and human infection is still uncertain.

2.3 Colonization of the live bird

Food safety is an international responsibility that encompasses all nations and societies of the world. Each country and region has it own unique food safety problems that relate to culture, climate, economic status and many other factors. However, the same bacterial enteropathogens, *Salmonella, Campylobacter, Clostridium perfringens* and *Listeria monocytogenes*, are common to all raw poultry and red meat.

The intestinal tract of poultry is complex and multi-compartmental, and is primarily concerned with the digestion and absorption of food materials. It also

provides a unique ecological niche for a broad variety of microorganisms, most of which exist in a commensal relationship with the host. Generally, foodborne pathogens are among the organisms that are carried by poultry as commensals and thus do not elicit defence responses in the bird. Factors affecting microbial colonization of the intestinal tract are many and varied. They include:

- ingestion of organisms with contaminated food and water
- recycling by coprophagy
- microbial survival during passage through the gastric barrier
- in the case of a specific organism, locating a favorable colonization site
- development of symbiotic interactions between microorganisms
- effective competition between an ingested organism and other bacteria
- nature of the host's diet
- physiological status of the host
- health and disease status of the host
- host age
- environmental stresses
- medication effects
- host genetic background.

Thus, microbes that successfully colonize the intestine do so in a highly complex, dynamic, competitive and interactive environment. In this regard, *Salmonella* has been studied more intensively than all of the other foodborne pathogens combined.

2.4 *Salmonella* and *Campylobacter* in poultry production

In the past, most efforts to control *Salmonella* in poultry have concentrated on processing; however, improving the microbiological quality of carcasses in the processing plant is difficult. Rather than eliminate *Salmonella*, the best the industry has been able to accomplish so far is to minimize cross-contamination. Therefore, to overcome the limitations of processing, *Salmonella*-free chickens must be delivered to the processing plant and control of *Salmonella* during the grow-out period is therefore critical. In practice, the situation is complicated, because there are so many potential sources of infection or contamination in an integrated poultry operation. These include chicks, feed, rodents, wild birds, insects, fomites, transportation and processing. If prevention of colonization or a significant reduction in *Salmonella* carriage is to be achieved during production, critical control points must be determined and techniques to control them implemented. Probably, the most critical factor is delivery of *Salmonella*-free chicks to the grow-out houses. This is based on the fact that, at hatch, most chicks have a very limited gut microflora and are far more susceptible to *Salmonella* colonization than older chicks. Milner and Shaffer (1952) first observed that colonization of chicks was dose-dependent and varied with day of challenge. They found that day-old chicks could be colonized by about ten

Salmonella cells, while later colonization was irregular and required higher challenge doses. Older chicks have a more mature gut microflora (Barnes *et al.*, 1972) and thus are more resistant to intestinal colonization by *Salmonella*. Feed and environmental sources still play a role in the transmission of *Salmonella* during chicken production, but the salmonellas that chicks can bring with them into the grow-out house must be controlled before 'competitive exclusion' treatment or other control measures can be expected to work (see Chapter 5).

Erwin (1955) first recovered viable *Salmonella* from commercial poultry feed and, since that time, the role of feed and feed ingredients in the spread of *Salmonella* throughout the poultry industry has received a great deal of attention. However, other sources appear to contribute more to contamination of finished carcasses than feed itself. These include breeder flocks, hatcheries and litter. For example, Goren *et al.* (1988) showed that serotypes of *Salmonella* on processed carcasses were also found in hatchery samples, but were not isolated from the feed.

Since the early 1980s, *Campylobacter jejuni* has been acknowledged as the most common human enteropathogen in developed countries (Tauxe *et al.*, 1988; Mead *et al.*, 1999). Various potential sources of infection have been identified, including contaminated food and water, and domestic pets. Untreated water (Tauxe *et al.*, 1982) and unpasteurized milk (Blaser, 1984) have accounted for most of the outbreaks, with large numbers of associated cases, but most cases are sporadic and it has been suggested that 20–40% of these might be due to the consumption of chicken (Report, 2004).

There are distinct differences in the ways in which chickens become infected with *Salmonella* and *Campylobacter* during production and with those in which populations of these pathogens are maintained. Unless there is disease or temperature stress, the highest levels of *Salmonella* colonization generally occur during the second or third week of grow-out, after which there is typically a gradual decline in the numbers until the time of slaughter. *Campylobacter,* on the other hand, is rarely found in chicks before the second or third week of grow-out, but, when colonization occurs in the house, practically all birds become colonized within only a few days and will remain colonized throughout the rearing period. Likely vectors include flies, wild birds, rodents and possibly untreated water. Recently, evidence has emerged to implicate breeders and fertile eggs as potential sources of *Campylobacter* infection for subsequent broiler offspring. Genotype analyses of *Campylobacter* strains isolated from commercial broiler-breeder flocks and from their respective progeny have demonstrated that the isolates from these epidemiologically-related sources were clonal in origin (Pearson *et al.*, 1996; Cox *et al.*, 2002b). In addition, *Campylobacter* has been isolated from the reproductive tracts of healthy laying hens and broiler breeders (Jacobs-Reitsma, 1997; Camarda *et al.*, 2000; Buhr *et al.*, 2002; Hiett *et al.*, 2002b), as well as from the semen of commercial broiler-breeder roosters (Cox *et al.,* 2002a). Even more convincing, *Campylobacter* has been detected by traditional culture methods in hatchery debris (Bennett, 2001; Bates, 2002) and PCR was capable of detecting *Campylobacter* DNA, both in

these hatchery samples (Hiett *et al*., 2002a) and the intestinal tracts of developing embryos (Hiett *et al.*, 2003). As mentioned earlier, *Salmonella* is usually found in low numbers (< 100 cfu) on broiler carcasses leaving the processing plant, whereas *C. jejuni* is often found in much higher numbers, more than 10^3 cfu per carcass (Waldroup *et al.,* 1992). As with *Salmonella*, it is likely that reductions in carcass contamination with *C. jejuni* can only be achieved by delivering broilers to the plant that are *Campylobacter*-free or at least have significantly reduced levels of the organism.

2.5 Other pathogens

Clostridium perfringens is recognized as an enteric bacterial pathogen for humans, poultry, other domestic animals and wildlife worldwide (Songer, 1996). The organism has been isolated from processed broiler chickens and samples taken in the processing plant (Mead and Impey, 1970; Lillard, 1971; Craven, 2002). It is reported to be a significant cause of foodborne disease outbreaks arising from the consumption of contaminated poultry and other meat products (Bean *et al.*, 1996). Little is known about the distribution and sources of *Cl. perfringens* in poultry-production facilities. In one study, *Cl. perfringens* was detected in a high proportion of paper pads used to line chick transport trays and collected after transport of chicks from the hatchery to the farm (Craven *et al.*, 2001b). Some of the isolates from the paper pads belonged to the same ribotypes as those of isolates from associated broiler carcasses (unpublished data). In another study, *Cl. perfringens* was isolated from shell fragments and chick fluff collected from the hatchers in broiler hatcheries (Craven *et al*., 2001a), indicating that chicks can become colonized at an early stage.

While *Listeria monocytogenes* and *Yersinia enterocolitica* have been isolated readily from processed broiler carcasses, very little is known about the ways in which these microbes enter poultry operations. However, *L. monocytogenes* was isolated from 1% of broiler hatchery samples (Cox *et al*., 1997), again suggesting the possibility of early chick exposure.

2.6 Possible control strategies

Salmonellosis in poultry is caused by either the host-adapted serotypes, such as *S. gallinarum* and *S. pullorum,* that cause disease in the birds, or by non-host-adapted types, which are usually pathogenic for man. Eradication programs for *S. gallinarum* and *S. pullorum* have been highly successful and, on the whole, they have resulted in the elimination of these serotypes from the poultry industry in the USA. In the vast majority of cases, human beings acquire *Salmonella* by the ingestion of contaminated food or water. Poultry (chickens, turkeys and ducks) and poultry products (particularly eggs) are among the most important sources of human infection and are estimated to be responsible for about 50% of

common-vehicle outbreaks. The development of vaccines to prevent human and poultry diseases has been a major accomplishment in the field of immunology. Some diseases have been eliminated through the use of vaccines. However, while existing vaccines have certainly diminished the incidence, morbidity and mortality of a large number of infectious diseases, salmonellosis is still very difficult to prevent or control in domestic animals through immuno-prophylaxis.

In the age of antibiotics, why should anyone attempt to develop a bacterial vaccine? Vaccines are needed, even with available antibiotics, because antibiotic treatment of many bacterial infections cannot prevent serious sequelae. Also, evolution of drug-resistant bacteria reduces the beneficial effects of antibiotics. The use of bacterins and attenuated, live cultures as vaccines for the prevention of avian salmonellosis has been examined experimentally, but has never had wide application under field conditions in the USA, until recently.

Prophylactic vaccination is also a possible means of preventing vertical transmission of *Salmonella*. For the large-scale use of prophylatic vaccines, they must be both safe and effective. The effectiveness of these vaccines may vary with the method of preparation. Adjuvants of many types from alum, through oil emulsions, to polynucleotides, have been used in relation to a variety of diseases that are controlled with prophylatic vaccines. An ideal vaccine should mimic the immunological stimulation associated with the natural infection, evoke minimal side effects and be readily available, cheap, stable and easily administered.

2.7 Future trends

Assurance of microbiological food safety for fresh poultry products is complicated by the many stages involved in production and processing. The most effective way to minimize the public health hazards associated with infectious agents in poultry is to initiate intervention strategies throughout the farm-to-fork continuum. There are numerous potential sources of contamination in an integrated poultry operation and, since multiple entry points exist for foodborne pathogens, multi-faceted intervention approaches are required during the various phases of grow-out and processing. Recent research suggests that pre-harvest pathogen reduction is a practical possibility and is important because the pathogen load on poultry at slaughter can, in turn, affect the microbiological status of the processed carcass.

In the USA, the main focus of the so-called Mega-Reg is the application of the HACCP concept in the processing plant, while the current emphasis in Europe is more towards on-farm control. In fact, over the last decade, there have been few, if any, technological developments in processing that have improved the microbiological status of processed products. The present situation is unlikely to change in the near future, because there is no financial incentive for processors to adopt new systems. However, decontamination of the end-product remains an attractive option, if an effective treatment can be developed that does not damage product quality and is equally acceptable to all stakeholders. For this

purpose, a physical treatment would be preferable to a chemical one and the possible use of microbiological agents, such as bacteriocins or bacteriophage, is also being investigated.

At farm level, there needs to be greater emphasis on upgrading present standards of biosecurity and identifying those hygiene measures that are most effective in controlling foodborne pathogens. This applies particularly to *Campylobacter*, for which sources and routes of transmission to poultry flocks are not yet well understood. Whatever the situation for pathogen control in ten years' time, there is no real prospect that pathogen-free poultry meat can be guaranteed, and there will still be a requirement for due care on the part of those responsible for cooking and handling the product prior to consumption.

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3

Detecting and controlling veterinary drug residues in poultry

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3.1 Introduction

Veterinary drugs have become an integral part of animal husbandry. They are used not only for the prevention and treatment of animal diseases, but also for growth-promoting purposes. The controlled usage of veterinary drugs is a prerequisite for the well-being of the animal and also for the quality and safety of human food. However, there is strong evidence that unwanted drug residues occur in animal products, and consumers may be exposed to harmful concentrations of residues. Consequently, the only way to guarantee food safety in this respect is to test foodstuffs for the presence of various drug residues. A few decades ago, food was considered safe, if no potentially harmful residues were found. This was easily accomplished, as the analytical methods were not particularly sensitive at that time. Now, with the constant improvements in analytical methods, it is no longer realistic to expect food to be completely free from residues.

Controlling veterinary drug residues has become an important issue in the food safety policies of the European Union (EU). In the EU, veterinary medicinal products (veterinary drugs) are defined as 'any substance or combination of substances presented for treating or preventing disease in animals or which may be administered to animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in animals' (Anon., 2001a). The EU has obliged the Member States to monitor food-producing animals for a wide range of veterinary drugs in order to protect consumer health (Anon., 1996). Directive 96/23/EC stipulates that a certain proportion of the total annual production of animal food commodities should be

monitored for various residues. In each Member State, the number of samples to be analysed is linked to the animal production figures for the preceding year. For example, in the case of poultry, the minimum number of samples taken each year must be at least one per 200 tonnes of the annual production, with some supplementary stipulations, and, for bovine animals, the testing rate should be at least 0.4% of the number of animals slaughtered the previous year. To guide the 'hunt for residues', the authorities have established maximum residue limits (MRLs) for various veterinary drugs. An MRL is defined as 'the maximum concentration of residue legally permitted to be present in a certain edible tissue'. For example, in the EU, the MRL for the antibiotic doxycycline is set at 100 μg per kg in poultry muscle and 300 μg per kg in the liver. The concepts of 'no observed effect level' (NOEL) and 'acceptable daily intake' (ADI) are used as the basis for the determination of MRLs. In addition, withdrawal periods have been established to protect consumers from unwanted residues in food. A withdrawal period is the time that passes between the last administration of a drug to an animal and the point at which the levels of residues in the tissues (muscle, liver, kidney, skin/fat) or products (milk, eggs, honey) fall below the stated MRL. Until this time has elapsed, the animal, or its products should not be used for human consumption.

Monitoring of residues has proved to be challenging, due to the broad range of legal and illegal chemical substances that are used in animal husbandry. Drugs can be metabolised rapidly to various compounds that may actually be more toxic than the parent drug itself. Therefore, it is sometimes necessary to analyse both the parent drug and the metabolite. Drugs can also form conjugates, and there might be a need to differentiate between the 'free' and 'bound' proportions of the drug. Furthermore, endogenous compounds may sometimes interfere with the analysis. To further complicate the monitoring, there are no methods available for some compounds, and the existing methods may sometimes be too complex or expensive for use in routine analysis.

The following sections give an introduction to the general principles of sample preparation and the methods used in drug residue analysis. Due to the limited space, the focus will be on antibacterials and anticoccidials, which are, perhaps, the most widely utilised classes of veterinary drugs in poultry production.

3.2 Sample preparation techniques

3.2.1 General principles

In food analysis, and especially in the area of drug residue analysis, sample preparation plays an essential role. The need to prepare samples inevitably slows down the analysis; in fact, it has been estimated that, on average, approximately 50–75% of the analysis time goes on sample pre-treatment. Therefore, the central aim in developing new techniques is to simplify the sample preparation protocols.

Storage conditions and handling of the sample can have a critical effect on the stability of the analyte. The composition of the sample may change with time, due to internal chemical changes, reactions with light, etc. In general, sample matrices, such as egg yolk or a portion of liver, are not suitable for analysis, as such. Therefore, tissue and egg samples are homogenised mechanically prior to analysis, after which the drug residues typically have to be extracted from the homogenised matrix. On the other hand, liquid samples, such as blood, plasma and urine, may need only a dilution step in order to be analysed directly (Bacigalupo *et al.*, 1995; Elliott *et al.*, 1996; Crooks *et al.*, 1998b). The purpose of the extraction is to recover the analyte and exclude all interfering substances that may co-extract with the analyte and distort the detection, identification and quantification. Unfortunately, a universal extraction strategy cannot be developed, since the extraction protocol is greatly dependent on the drug in question and its concentration, the sample matrix and the analytical method. To further eliminate interfering compounds, an additional clean-up step is normally needed, after preliminary extraction. Traditionally, this is achieved by liquid-liquid partitioning, which separates the drug from other matrix components by partitioning the residue of interest between two immiscible phases (generally an organic and an aqueous phase). For example, lipids are usually removed with n-hexane from acetonitrile extracts (Kennedy *et al.*, 1995b; Schneider and Donoghue, 2002). The use of solid-phase extraction (SPE) has become nearly ubiquitous as a part of the basic methodology for extract clean-up. SPE is considered to be better than traditional solvent partitioning, which is prone to operator-dependent errors. In addition, the SPE step is easier to automate and the extracts are cleaner (deZeeuw, 1997).

Typically, quantitative and confirmatory methodologies, such as chromatographic or mass-spectrometric methods, require extensive treatment of extracts. Poole *et al.* (1990) have given a thorough review of the sample preparation needed for chromatographic separations. On the other hand, rapid screening methods based on immunochemistry or microbiology may require only minimal sample preparation. For instance, simple, extraction protocols for egg samples, using acetonitrile as the organic solvent and either sonication (Hagren *et al.*, 2004, 2005) or shaking (Peippo *et al.*, 2004) to aid the transfer of the drug to the organic phase, without any further purification steps, have been reported recently.

3.2.2 Sample preparation for antibiotic residues in poultry

The general principles of sample preparation for the analysis of antibiotic residues in poultry tissues are presented in Fig. 3.1. After preparing a representative, homogenised sample, a suitable solvent, which is typically acetonitrile, chloroform, ethyl acetate or ether, is added for extraction purposes. The extract is separated from the remains of the tissue by centrifugation or filtration, and a de-fatting solvent, such as hexane or iso-octane, may be added to

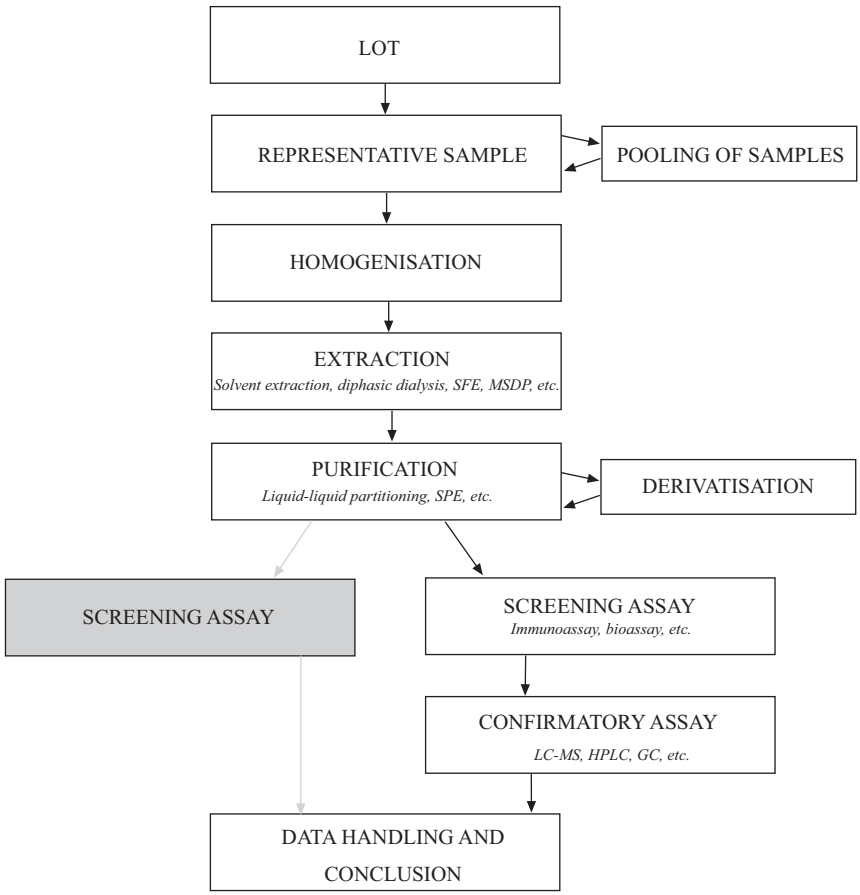

Fig. 3.1 Flow chart presenting a typical sample of preparation protocol.

re-extract the residues. Further clean-up is usually performed by means of liquid–liquid partitioning or SPE, as discussed earlier. The extract is usually concentrated by evaporation to a smaller volume, to achieve the required limit of detection (LOD), and derivatised if needed. Finally, the extract can be screened in an immunoassay, etc., and then, when necessary, subjected to confirmatory testing.

Organic solvents are commonly employed in the extraction of antibiotic residues from tissues. The term solvent extraction refers to the transfer of the analyte from the tissue to the solvent. Desorption of drugs from the tissue matrix depends on the molecular interactions between the drug and the matrix. Organic solvents are selected, according to the properties of the analyte, by empirical or thermodynamic techniques. However, the solvents used should not harm the analyte. In general, polar drug residues are best extracted by using polar solvents. Salvatore and Katz (1993) determined the solubilities of several antibiotics in various organic solvents, and found some common trends. The

solubility of macrolides, tetracyclines and peptide antibiotics was increased as the solvent polarity increased, whereas aminoglycosides, neomycin and streptomycin, were poorly soluble in all solvents. Dimethyl sulfoxide and methanol were found to be the best 'all-purpose solvents' for antibiotics.

In order to reduce the amounts of hazardous organic solvents used, compensatory methods for traditional solvent extraction have been developed. Recently, a diphasic dialysis system was utilised in the extraction of enrofloxacin and ciprofloxacin from eggs (Lolo *et al.*, 2003). Diphasic dialysis is based on liquid-liquid extraction, which enables the transfer of low molecular mass analytes from aqueous phase to non-polar organic phase. The technique enables residues to be extracted directly from foodstuffs, and there is no need for any complementary purification steps. Kubala-Drincic *et al*. (2003) described a matrix solid-phase dispersion (MSPD) technique for chloramphenicol in poultry muscle tissue. MSPD utilises bonded-phase solid supports as an abrasive to disrupt the sample and as a bound solvent to aid complete disruption during the blending process (Boyd *et al.*, 1994). Therefore, this technique allows sample homogenisation, destruction of cellular structure, extraction and clean-up in a single procedure. Increasingly, MSPD has been applied to the preparation of different biological samples for the analysis of residues and contaminants (Barker and Long, 1989, 1992; Schenck *et al.*, 1992; LeBoulaire *et al.*, 1997). Supercritical fluid extraction (SFE) is another alternative for traditional solvent extraction. Pensabene *et al.* (1999) reported the application of SFE, using supercritical carbon dioxide without a solvent modifier, for the isolation of chloramphenicol residues from egg products. Shim *et al.* (2003) described the use of SFE for enrofloxacin residues in chicken muscle. However, it is not yet used widely, due to the requirement for specialised equipment. The theory of antibiotic extraction from biomatrices has been reviewed by Fedeniuk and Shand (1998).

3.3 Analytical methods for drug residues

This section focuses on antibacterials and anticoccidials. A brief introduction to the characteristics of each class is given, and various analytical methods, particularly for poultry tissues and eggs, are presented. Typically, several methods may exist for a certain compound, but only a few have been validated for use on poultry products. The purpose of this chapter is not to provide a thorough cross-section of all the available methods for residue analysis, but rather to give some general ideas and references that can be used as a starting point in a search for additional information.

3.3.1 Antibacterials

Antibacterials are used in animal husbandry for the treatment and prevention of various bacterial diseases and for growth enhancement. The term 'antibacterial' actually covers two different classes of drugs: antibiotics, which are natural

products, and antibacterials, which are synthetic compounds. However, both of these classes possess antibacterial activity. Antibacterials can be classified according to their source, biological properties, mode of action or chemical structure. The classification based on chemical structure is applied in this section.

Microbiological inhibition tests have been utilised traditionally in the screening of various antibiotic residues. These tests are based on the ability of antibiotics to inhibit the growth of sensitive bacteria. However, simple inhibition tests, using only one test strain and medium, cannot differentiate between individual antibiotics. Consequently, the sample may have to be subjected to a group-specific test and/or chromatographic identification, which make the system very time-consuming. On the other hand, microbiological assays are inexpensive and require only simple equipment. Therefore, they can be used for the screening of large numbers of samples. In addition, several thin-layer chromatographic methods have been developed. However, nowadays, systems based on liquid chromatography (LC) are usually preferred for the detection of antibiotic residues in foodstuffs. Reviews by Kennedy *et al.* (1998a) and Niessen (1998) provide detailed information on the use of LC–mass spectrometry (MS) in antibiotic analysis. Since gas chromatography (GC) is suitable only for volatile (naturally-occurring or derivatised) compounds, the technique is not so commonly used for antibiotic residues. However, a GC method, in which chloramphenicol was derivatised with trimethylsilyl, was reported recently (Kubala-Drincic *et al.*, 2003). In addition, several immunoassays have been developed for antibiotics.

Aminoglycosides and aminocyclitols

Aminoglycosides and aminocyclitols are antibiotics produced by bacteria belonging to the genera *Streptomyces* and *Micromonospora*. Aminoglycosides usually contain two or more amino-sugars that are linked with glycosidic bonds to a central aglycon component. Aminocyclitols, such as spectinomycin, are closely related structurally to aminoglycosides. In food-producing animals, streptomycin, dihydrostreptomycin, gentamicin, neomycin and spectinomycin are commonly used for the treatment of bacterial infections. They act by binding to the ribosomes of Gram-negative and some Gram-positive bacteria, thereby inhibiting protein synthesis. Stead (2000) reviewed the methods employed specifically for the analysis of aminoglycosides. In addition, reviews focusing on LC–MS methods for the determination of aminoglycosides have been published (Kennedy *et al.*, 1998a; Niessen, 1998).

The case of spectinomycin is now considered. This is an aminocyclitol antibiotic produced by *Streptomyces spectabilis*. Spectinomycin is effective against Gram-negative bacteria, but its activity against Gram-positive bacteria is limited. The antibiotic has low toxicity, which is a beneficial characteristic. The drug is usually administered to animals via oral or intramuscular routes to treat respiratory diseases. In the EU, the MRLs for spectinomycin residues in all food-producing species have been set at 5000, 1000, 500, 300 μg per kg for kidney, liver, skin/fat and muscle, respectively (EMEA, 2002). No MRL has been established for eggs, since spectinomycin is not licensed for use in layers.

Since there is no chromophore in spectinomycin, it cannot be detected with an ultraviolet (UV) detector, without derivatisation. A high-performance liquid chromatography (HPLC) method involving post-column oxidation and *o*-phthaldialdehyde derivatisation was described for chicken plasma samples, with a limit of quantification (LOQ) of 0.06 μg per ml (Haagsma *et al.*, 1995). Another approach, based on immunochemistry, namely an enzyme-linked immunosorbent assay (ELISA) for chicken plasma, was described with a LOQ of 0.05 μg per ml (Tanaka *et al.*, 1996). Bergwerff *et al.* (1998) developed an HPLC–fluorescence method for chicken egg, liver, muscle and fat, using acidic buffer and dichloromethane for primary extraction and SPE for further sample clean-up. Concentrations of 50 μg per kg and above could be detected with this system, which is very adequate, considering the current MRLs. Another technique using an HPLC coupled with an atmospheric-pressure, chemical-ionisation tandem MS, was validated at a concentration level of 100 μg per kg, but only bovine tissues were used as the sample matrix (Hornish and Wiest, 1998).

Amphenicols

Chloramphenicol, thiamphenicol and florfenicol are broad-spectrum antibacterials that have very similar chemical structures. Thiamphenicol and florfenicol have MRLs ranging from 50 to 2500 μg per kg. Chloramphenicol has been widely used in animal husbandry for the treatment of various infections. However, its use is currently prohibited in many countries, because of its proved toxicity to humans (Settepani, 1984). Relatively low levels of chloramphenicol may give rise to an irreversible type of bone-marrow depression that can lead to aplastic anaemia and Gray's Syndrome (Feder *et al.*, 1981). The toxic effects are not dose-dependent, but rather related to the hypersensitivity of certain individuals.

Chloramphenicol is characterised chemically by a benzene ring and a substituted three-carbon chain. Since it is highly polar and forms glucuronates in liver and kidney, tissue samples require special handling techniques (Cooper *et al.*, 1998b). Reports in the literature indicate that enzymatic digestion is necessary to liberate bound chloramphenicol residues from the glucuronide conjugate, when examining liver samples. Mottier *et al*. (2003) analysed poultry meat, with and without incorporation of the enzymatic digestion step. Similar results were obtained, which confirmed the absence of glucuronidation in chicken muscle. Therefore, the digestion step could be omitted with poultry muscle samples. The extraction of chloramphenicol is usually performed with acetonitrile or ethyl acetate. Clean-up by SPE, using polar and non-polar materials, has been utilised in sample preparation. Chloramphenicol could also be extracted from eggs by using supercritical carbon dioxide without a solvent modifier (Pensabene *et al.*, 1999). A study of the accumulation of chloramphenicol in eggs showed that residue content in yolk was considerably higher than in albumen (Akhtar *et al.*, 1995). In addition, the residues in yolk appeared to persist, which might be associated with the lipid content of yolks. The results

also indicated that no loss of chloramphenicol occurred during the freeze-drying of eggs. Ramos *et al*. (2003) conducted a stability study for chloramphenicol and found that the residues were stable in muscle during refrigeration and freezing. Taken together, these studies suggest that chloramphenicol residues are rather stable in those matrices and samples can be stored safely for a relatively long period.

Several methods, based on various analytical techniques, are described in the literature for the determination of chloramphenicol residues in foodstuffs. Fewer methods have been reported for other amphenicols (Nagata and Saeki, 1991, 1992). Most commonly used methods for the determination of chloramphenicol in tissues and eggs include GC (van Ginkel *et al.*, 1990; Mineo *et al.*, 1992; Akhtar *et al.*, 1995; Gude *et al.*, 1995) and LC (Delepine and Sanders, 1992; Hummert *et al.*, 1995; Hormazabal and Yndestad, 2001; Li *et al.*, 2002). Kubala-Drincic *et al*. (2003) employed MSPD prior to detection of chloramphenicol residues in muscle tissue by GC with an electron-capture detector and a mass spectrometer. Borner *et al.* (1995) used GC-MS for the analysis of chloramphenicol residues in eggs. For confirmatory purposes, MS is generally accepted as the detection technique of choice. Mottier *et al*. (2003) described a confirmatory method for chloramphenicol in poultry muscle, using LC–electrospray ionisation (ESI) tandem MS. The method was quantitative and entailed liquid–liquid extraction with ethyl acetate–diethyl ether, followed by an extensive clean-up step with SPE. The decision limit CCα (0.01 μg per kg) and detection capability CCβ (0.02 μg per kg) were established according to the EU Directive (2002/657/EC).

To sum up, the analytical techniques for chloramphenicol residues have evolved as different technologies have become available. Sample purification steps have largely been supplanted by SPE. Techniques such as MSPD, where the initial, liquid-solubilisation of the drug is replaced by the use of a solid support, have also been reported. The analytical methods are mostly based on chromatographic techniques.

β-lactams

β-lactams are chemically characterised by a β-lactam ring connected to a thiazolidine ring (penicillins) or a dihydrothiazine ring (cephalosporins and cephamycins). All β-lactams are bacteriocidal and their activity against Gram-positive and Gram-negative bacteria is based on their ability to interfere with the development of bacterial cell walls. Representatives of the penicillin class are penicillin G (a.k.a. benzylpenicillin), amoxicillin and ampicillin. In veterinary practice, penicillins have been used extensively for decades for all food-producing species. The MRLs in the EU for penicillins range from 4 to 300 μg per kg, depending on the compound and sample matrix.

Analytical methods for β-lactams are typically based on microbiological inhibition assays or LC separation combined with UV, fluorescence or MS detection. GC–MS is a feasible combination for confirmatory purposes, but only after derivatisation of β-lactam residues. In general, β-lactams are unstable in

organic solvents, which may bias the analysis. Therefore, traditional extraction methods have utilised aqueous extractions with protein-removing agents in order to obtain cleaner extracts, and SPE and immuno-affinity cartridges have been used for subsequent clean-up steps. A short review of the extraction methods for β-lactams is given by Fedenuik and Shand (1998). Most reported methods for β-lactams have used bovine tissues or milk, not poultry tissues, as samples. However, Furusawa (2001) reported a simple and rapid HPLC method for the determination of residual penicillin G in chicken tissues. Sample preparation was performed by extracting penicillin G with 0.1 M phosphate buffer, which was followed by ultrafiltration of the extract. A photodiode array detector (DAD) was used for the detection, and the LOD was determined as 40 μg per kg. A sensitive HPLC method, with fluorescence detection for ampicillin residues in chicken tissues, was also described (Luo *et al.*, 1997). The sample extraction protocol was extremely simple: after homogenisation, the proteins were precipitated with trichloroacetic acid. The sample was then centrifuged and the supernatant filtered prior to analysis by HPLC. The mean recoveries were >90% at three levels. The concentration LOD and LOQ were 0.6 and 1.5 μg per kg, respectively, which was very adequate in view of the present MRLs. Reviews covering the use of LC-MS in β-lactam analysis have been produced by Kennedy *et al.* (1998a) and Niessen (1998).

Macrolides

Macrolide antibiotics, such as spiramycin, tylosin and erythromycin, are composed of 12–16-membered lactone rings, to which sugar moieties are attached. Macrolides are produced by various *Streptomyces* strains and they are active against Gram-positive bacteria. However, their activity is limited against Gram-negative bacteria. Macrolides are used in veterinary practice to combat various infectious diseases. Residues of these antibiotics in edible tissues can lead to allergies in sensitive individuals or bacterial resistance. In the EU, MRLs vary from 75 to 1000 μg per kg, depending on the target tissue (see Table 3.1).

Reviews by Fedenuik and Shand (1998), Kennedy *et al.* (1998a) and Kanfer *et al.* (1998) deal with the extraction and analytical methods for macrolides. Traditionally, alkali-treated or aqueous tissue homogenates are extracted with organic solvents and further purified by back extraction into weakly acidic,

Table 3.1 Summary of the current MRLs for macrolides in the EU

Compound	MRLs (μg per kg)				
	Muscle	Fat	Liver	Kidney	Eggs
Erythromycin	200	200	200	200	150
Spiramycin*	200	300	400	—	—
Tilmicosin	75	75	1000	250	—
Tylosin	100	100	100	100	200

* Sum of spiramycin and neospiramycin.

aqueous buffers. However, macrolides, especially erythromycin, are often unstable in acidic solutions, and almost all macrolides are converted to degradation products at pH < 4.5. Various reported methods have used solvents, such as acetonitrile (Juhel-Gaugain *et al.*, 1999), acidic methanol (Leal *et al.*, 2001; Codony *et al.*, 2002; Horie *et al.*, 2003) and chloroform and ethyl acetate (Prats *et al.*, 2002) for the extraction of macrolides from poultry tissues.

Liquid chromatographic methods allowing multi-residue analysis have been described for the quantitative analysis of macrolides (Horie *et al.*, 1998, 2003; Kees *et al.*, 1998; Juhel-Gaugain *et al.*, 1999; Leal *et al.*, 2001; Codony *et al.*, 2002; Prats *et al.*, 2002). Table 3.2 lists some of the methods that have been developed specifically for poultry tissues and eggs. Although UV absorption is the most commonly used detection system (Horie *et al.*, 1998; Juhel-Gaugain *et al.*, 1999; Leal *et al.*, 2001), some macrolides lack a suitable chromophore group, and, therefore, electrochemical detectors (Kees *et al.*, 1998) or fluorescence-based systems (Leroy *et al.*, 1994) via pre-column derivatisation have been developed. Leal *et al*. (2001) reported an LC method using UV-DAD detection for the determination of seven macrolides: spiramycin, tilmicosin, oleandomycin, erythromycin, tylosin, kitasamycin and josamycin. The method proved to be applicable to five of the macrolides studied in poultry muscle, but it lacked the required sensitivity for erythromycin and oleandomycin. A quantitative LC–electrospray–MS method was reported that was capable of determining seven macrolides in spiked chicken tissues (Codony *et al.*, 2002). The LODs for these macrolides were typically < 4 μg per l, except for spiramycin and tilmicosin, which had LODs of 35 and 8 μg per l, respectively. Horie *et al*. (2003) reported a rapid and reliable method for the simultaneous determination of eight macrolides in poultry meat by LC–ESI–MS at a level of 10 μg per kg.

In conclusion, liquid chromatographic systems are commonly used in the analysis of macrolide residues in foodstuffs. Multi-residue analysis can be accomplished by an LC combined with MS. Sample preparation is usually based on solvent extraction and clean-up is performed either by SPE or liquid–liquid partitioning. Immunoassays for macrolide residues in poultry tissues or eggs have not been reported, but a particle-concentration fluorescence immunoassay for the determination of tylosin in feed has been published (Wicker *et al.*, 1994).

Nitrofurans

Nitrofurans are compounds that have a common structure: a characteristic 5-nitro group. This group is responsible for their antimicrobial activity. Nitrofurans have been used as feed additives to treat bacterial infections e.g. in cattle, pigs and poultry. However, the use of nitrofurans is currently prohibited in the EU and USA, due to toxicological concerns and the consequent public health risk. Nevertheless, nitrofuran residues have been found recently in poultry and aquaculture products imported into the EU. Since nitrofurans are unauthorised compounds, analytical techniques should aim to reach the lowest possible limit of detection. Generally, MS is the method of choice for the unequivocal confirmation of banned residues. Because the parent drugs

Table 3.2 Summary of the methods used in macrolide analysis for poultry products

Reference	Analyte	Method	Matrix[a]	Pre-treatment	LOD (μg per kg)	LOQ (μg per kg)
Codony *et al.* (2002)	Multi-residue	LC-ESI-MS	M	Solvent extraction and SPE clean up	24–35[b]	
Horie *et al.* (2003)	Multi-residue	LC-ESI-MS	M	Solvent extraction and SPE clean up		10
Juhel-Gaugain *et al.* (1999)	Multi-residue	LC-UV	M	Solvent extraction and SPE clean up	15–30	
Leal *et al.* (2001)	Multi-residue	LC-UV	M	Solvent extraction and SPE clean up		30–2950[b,c]
Prats *et al.* (2002)	Tylosin	LC-UV	F, K, L, M, S	Solvent extraction		50

KEY: [a] F, fat; K, kidney; L, liver; M, muscle; S, skin. [b] μg/l. [c] values for buffer standards. For other abbreviations, see text.

(nitrofurantoin, nitrofurazone, furaltadone and furazolidone) are rapidly metabolised, methods capable of detecting the metabolites, which are stable and persistent in animal tissues, have proved to be useful.

Many methods for the analysis of nitrofuran residues in pigs have been reported (Horne *et al.*, 1996; McCracken and Kennedy, 1997; Leitner *et al.*, 2001; Conneely *et al.*, 2003). Recently, Cooper *et al.* (2004a, b) described the production of polyclonal antibodies for the metabolite of the nitrofuran furazolidone and the immunoassay for the screening of this metabolite in prawns. Only a few methods have been described for poultry (Kumar *et al.*, 1994; Draisci *et al.*, 1997). Draisci *et al.* (1997) described LC–UV and LC–MS methods for determining the parent drugs in eggs. The LODs were 2.5–5 μg per kg and 1–3.2 μg per kg, using UV and MS, respectively. Some preliminary reports on nitrofuran determination in turkey muscle and egg products were presented at the EuroResidue V, conference on residues of veterinary drugs in food (Kaufmann and Butcher, 2004; Yun *et al.*, 2004).

Peptide antibiotics

These compounds, such as bacitracin, avoparcin and virginiamycin, are large peptide molecules that often contain D-amino acids. They are similar to biological matrix components, which is one of the major reasons why peptide antibiotics are difficult to analyse from biological samples. Use of the above-mentioned peptide antibiotics as feed additives is currently prohibited in the EU. Analytical methods for bacitracin are now discussed.

Bacitracin is produced by certain strains of *Bacillus licheniformis* and *B. subtilis*. In fact, the various products generally referred to as 'bacitracin' are mixtures of similar polypeptides, which may differ by only one amino acid. To date, very few methods have been published on the analysis of food products for bacitracin. On the other hand, several methods exist for determining bacitracin in feed. Recently, biological and chemical methods used for bacitracin analysis were thoroughly reviewed by Sin and Wong (2003). An ELISA using horseradish peroxidase as a label enzyme and a simple dip-strip test permitted the monitoring of chicken plasma for bacitracin and was reported a few years ago (Matsumoto *et al.*, 1997). The antibody was specific for bacitracin and it did not exhibit cross-reactivity with other antibiotics used as animal feed additives or veterinary drugs. The LOD for bacitracin was estimated to be 0.1 μg per l and 50 μg per l with ELISA and the strip test respectively. The recoveries ranged from 97% to 103%.

Quinolones

Quinolones and fluoroquinolones are synthetic antibacterials, of which oxolinic acid, danofloxacin, enrofloxacin and norfloxacin are characteristic examples. Fluoroquinolones are second-generation quinolone derivatives, which contain a fluorine atom. The use of quinolones is not restricted to animal husbandry and they are also widely employed in human medicine. These antimicrobial agents are highly active against a broad range of bacteria. However, the emergence of

Table 3.3 Summary of the current MRLs for quinolones in the EU

Compound	MRLs (μg per kg)			
	Muscle	Fat	Liver	Kidney
Danofloxacin	200	100	400	400
Difloxacin	300	400	1900	600
Enrofloxacin	100	100	200	300
Flumequine	400	250	800	1000
Oxolinic acid	100	50	150	150
Sarafloxacin	—	10	100	—

drug-resistant bacteria has raised concerns about the use of such compounds and their potential impact on human health. Therefore, MRLs, which are summarised in Table 3.3, have been fixed for these drugs.

Solvents used for the extraction of quinolones from tissue samples include methanol–perchloric acid–phosphoric acid solution (Strelevitz and Linhares, 1996), acetone-chloroform-basic buffer (Eng *et al.*, 1998), dichloromethane (Barron *et al.*, 2001), acetonitrile–acetic acid (Rose *et al.*, 1998), acetonitrile–ammonia (Schneider and Donoghue, 2002) and acetonitrile basic solution (Yorke and Froc, 2000). The above-mentioned methods often utilised additional hexane washes or SPE for further sample purification. As an alternative method to traditional solvent extraction, SFE has been used for the pre-treatment of muscle samples containing enrofloxacin residues (Shim *et al.*, 2003), and diphasic dialysis has been employed for egg samples (Lolo *et al.*, 2003).

Various methods for the determination of quinolone residues in food products, especially fish, have been reported (Horie *et al.*, 1993; Schneider *et al.*, 1993; Doerge and Bajic, 1995). Several methods have also been developed for either mono-residue or multi-residue analysis of quinolones in poultry tissues and eggs, as shown in Table 3.4. Methods relying on a combination of solvent extraction, LC and fluorescence detection have been reported (Strelevitz and Linhares, 1996; Eng *et al.*, 1998; Rose *et al.*, 1998; Yorke and Froc, 2000). Many authors have also used MS in multi-residue analysis of quinolones in chicken tissues and eggs (Schneider and Donoghue, 2002, 2003; Lolo *et al.*, 2003). Schneider *et al.* (1993) described a method for the determination of danofloxacin residues in chicken liver by means of LC–MS–MS via an electrospray interface. The method allowed the confirmation of danofloxacin residues down to 50 μg per kg. An efficient multi-residue method for the detection of fluoroquinolone antibiotics in eggs was developed by Schneider and Donoghue (2003). Quantification and confirmation were achieved simultaneously by using LC in combination with fluorescence detection and multiple MS. With this method, eight fluoroquinolones could be analysed from inoculated egg samples, with recoveries in the range of 60–100% and LOQs of 0.1–2 μg per kg in whole eggs.

In addition, several non-chromatographic methods have been reported for quinolone antibiotics in poultry products. Capillary electrophoresis (CE) has

Table 3.4 Summary of the methods used in quinolone analysis for poultry products

Reference	Analyte	Method	Matrix	Pre-treatment	LOD (μg per kg)	LOQ (μg per kg)
Al-Mustafa and Al-Ghamdi (2000)	Norfloxacin	Microbiol.	M, L			
Barron *et al.* (2001)	Enrofloxacin	CE-UV-DAD	M	Solvent extraction and SPE clean-up	10	25
	Ciprofloxacin				<25	50
Barron *et al.* (2002)	Difloxacin	CE-UV-DAD	M	Solvent extraction and SPE clean-up	10	25
	Sarafloxacin				25	50
Chen and Schneider (2003)	Enrofloxacin	Spectrofluor.	M	Solvent extraction	300	
Donoghue and Schneider (2003)	Enrofloxacin	Microbiol.				
Eng *et al.* (1998)	Flumequine	LC-Fluor.	L	Solvent extraction and on-line dialysis	5	
	Oxolinic acid				2.5	
Holtzapple *et al.* (1997)	Sarafloxacin	ELISA	L	Homogenisation in buffer and centrifugation	2	
Lolo *et al.* (2003)	Enrofloxacin	LC-MS	E	Diphasic dialysis		2
	Ciprofloxacin					4
Rose *et al.* (1998)	Multi-residue	LC-Fluor./UV	M, L, E	Solvent extraction and SPE clean-up		5–50
Schneider *et al.* (1993)	Danofloxacin	LC-MS^n	L	Solvent extraction		50
Schneider and Donoghue (2002)	Multi-residue	LC-Fluor./MS^n	M, L	Solvent extraction	0.1–2	
Schneider and Donoghue (2003)	Multi-residue	LC-Fluor./MS^n	E	Solvent extraction		0.1–2
Shim *et al.* (2003)	Enrofloxacin	LC-Fluor.	M	Supercritical fluid extraction		5
Strelevitz and Linhares (1996)	Danofloxacin	LC-Fluor.	M, L	Solvent extraction		10
Yorke and Froc (2000)	Multi-residue	LC-Fluor.	M	Solvent extraction	0.5–35	

KEY: E, eggs; L, liver; M, muscle; MS^n, multiple mass spectrometry. For other abbreviations, see text.

become a useful tool in veterinary drug-residue analysis, because of its high resolution, speed and small sample volume requirement. The combination of the separation capability of CE with SPE improves CE sensitivity and thereby lowers the limits of detection and quantification. Barron *et al*. (2001) reported a method for the determination of enrofloxacin and ciprofloxacin in poultry muscle, using capillary electrophoresis. The same authors published another CE technique for difloxacin and sarafloxacin in poultry tissue (Barron *et al.*, 2002). The LODs obtained were low enough to determine concentrations below the permissible MRL. Chen and Schneider (2003) reported a simple spectrofluorometric method based on intrinsic enrofloxacin fluorescence. In this screening method, the separation of enrofloxacin from poultry tissue was accomplished with a single-step extraction and centrifugation, without any further clean-up. The method provided relatively reproducible results. However, reliable screening could be performed only at a quite high concentration level of 300 μg per kg. Also, microbiological and immunochemical screening methods offer proper alternatives to the conventional chemical methods. A microbiological method for the screening of norfloxacin in chicken muscle and liver was reported and this required no sample preparation, since a piece of tissue could be used as such for analysis on an agar plate (Al-Mustafa and Al-Ghamdi, 2000). Another microbiological method, using *Klebsiella pneumoniae* as an enrofloxacin indicator organism for egg samples, was reported recently (Donoghue and Schneider, 2003). Holtzapple *et al*. (1997) described an ELISA with a simple sample preparation protocol for sarafloxacin residues in chicken liver.

To conclude, LC is the most frequently employed analytical method to determine the presence of quinolone residues in poultry products. Typically, chromatographic techniques enable multi-residue analysis to be carried out, whereas, with non-chromatographic techniques, such as ELISA and CE, fewer compounds are detected.

Sulfonamides

Sulfonamides (sulfa antibiotics) are a class of synthetic molecules that share a common *p*-aminobenzenesulfonamide moiety, which is needed for the antibacterial activity. Representatives of this class are sulfadoxine, sulfadiazine, sulfaquinoxaline and sulfamethazine, which is, perhaps, the most extensively used sulfonamide. Sulfonamides function as veterinary drugs in the treatment of bacterial infections and as feed additives in growth promotion. In addition, sulfonamides have been utilised in the treatment and prevention of coccidiosis. It has been estimated that sulfa residues may cause unwanted symptoms in approximately 5% of human patients treated with sulfonamides; thus, the occurrence of sulfa residues in foodstuffs can pose a health risk for consumers. Moreover, the utilisation of milk contaminated with sulfa residues can be compromised, since the growth of bacteria needed in the production of yoghurt is inhibited. The MRL for sulfa residues in all target tissues is set at 100 μg per kg in the USA and the EU (Anon., 1999). However, in Japan, the MRL is 20 μg per kg.

Typical methods for the analysis of sulfa residues in food products include microbiological inhibition tests (Murphy *et al.*, 1986; Korsrud *et al.*, 1998) and various chromatographic methods (Jennings and Landgraf, 1977; Abian *et al.*, 1993; Simeonidou *et al.*, 1996; Abjean, 1997; Combs *et al.*, 1997; Tarbin *et al.*, 1999; Dost *et al.*, 2000; Fuh and Chan, 2001; Kishida and Furusawa, 2001; Heller *et al.*, 2002; Bogialli *et al.*, 2003), of which some have been developed specifically for poultry products. Tarbin *et al.* (1999) developed two methods based on GC–mass selective detection (MSD) and LC–MS that were suitable for the analysis of 15 sulfa residues in eggs. Prior to the gas chromatographic analysis, the residues had to be derivatised with diazomethane and penta-fluoropropionic acid anhydride. Generally, it is very difficult to develop a universal extraction protocol that can extract all members of a certain class of drug with similar efficacy. This was seen in recoveries at a concentration level of 100 μg per kg, which varied from 44 to 92% with LC–MS and from 54 to 110% with GC–MSD. Similar effects could also be seen in the study by Heller *et al*. (2002), who developed an LC–tandem MS method for confirmation and an LC–UV method for the quantification of 15 sulfa residues in eggs. Only one type of SPE column was used in the sample clean-up, whereas Tarbin *et al.* (1999) used both cation- and anion-exchange SPE columns. However, some additional centrifugation and evaporation steps were included in the sample preparation scheme by Heller *et al*. (2002). Therefore, the time spent in sample preparation was quite similar in both studies. The recoveries reported by Heller *et al.* (2002), using samples spiked at a concentration of 100 μg per kg, were in the range of 54–100% and thus were comparable with the results obtained by Tarbin *et al*. (1999). Both methods were capable of detecting residue levels below the EU MRL.

Normally, immunoassays are very specific and recognise only one analyte. Accordingly, several immunoassays have been described for the screening of one type of sulfa compound (Heering *et al.*, 1998; Elliott *et al.*, 1999; Lee *et al.*, 2001; Spinks *et al.*, 2001). However, in some cases, it would be more useful to have an immunoassay that could detect several members of a particular drug class at the same time. In fact, immunoassays that use cross-reactive antibodies have been reported recently. A biosensor assay capable of detecting eight sulfonamides in chicken serum was described by Haasnoot *et al.* (2003a). Korpimäki *et al.* (2002, 2004) used antibody engineering in the development of a broad-specificity antibody that was able to detect 18 sulfonamides simultaneously in one reaction. The tested sample matrices were chicken serum, bovine milk and muscle. All 18 sulfas could de detected at concentrations below the current MRL in the EU.

Tetracyclines

Tetracycline antibiotics are administered to food-producing animals as veterinary drugs to combat respiratory and systemic infections. Tetracyclines prevent the growth of bacteria by inhibiting protein synthesis and they have broad-spectrum activity against Gram-positive and Gram-negative bacteria.

Members of the tetracycline class in routine use are oxytetracycline, tetracycline, chlortetracycline and doxycycline. Chlortetracycline, the oldest member of the class, and oxytetracycline are produced by *Streptomyces aureofaciens*, whereas tetracycline and doxycycline are produced semi-synthetically. The MRLs for the compounds range between 100 and 600 μg per kg, depending on the matrix (Anon., 1999). In the USA, oxytetracycline and chlortetracycline have also been licensed for growth-promoting purposes.

Perhaps the most common extraction procedure for tetracyclines is to use acidic buffers and a clean-up with SPE. In addition, a new approach has been introduced in the sample clean-up: the metal-binding capability of tetracyclines can be utilised in metal-chelate affinity chromatography (Farrington *et al.*, 1991; Croubels *et al.*, 1997; Cooper *et al.*, 1998a). In the scientific literature, most of the methods used to determine tetracycline residues are based on LC, with various detection systems, such as UV, fluorescence (Blanchflower *et al.*, 1989; McCracken *et al.*, 1995; Zurhelle *et al.*, 2000) and MS (Oka *et al.*, 1994, 1997; Blanchflower *et al.*, 1997b; Kennedy *et al.*, 1998b; Zurhelle *et al.*, 2000; Bruno *et al.*, 2002). Since tetracyclines are non-volatile compounds, gas chromatographic methods cannot be exploited without derivatisation. A comprehensive review of MS in the detection of tetracyclines was given by Oka *et al*. (1998). An automated analytical method for tetracycline, oxytetracycline and chlortetracycline and their metabolites, in eggs and chicken plasma, was reported a few years ago (Zurhelle *et al.*, 2000). Clean-up was performed with an automated ASTED system, which included both dialysis and SPE steps. LC–UV/fluorescence or LC–MS–MS were used for detection. The LOQ in eggs varied from 34 to 45 μg per kg, which relates favourably to the present MRLs. An HPLC-DAD method for determining tetracyclines in chicken muscle and eggs was described by De Ruyck *et al.* (1999). In this case, the LOQ ranged between 13 and 52 μg per kg for eggs and between 18 and 37 μg per kg for muscle. Additionally, changes in the residue concentrations in eggs and muscle during a specified time period were determined with the aid of feeding trials.

3.3.2 Anticoccidials

Anticoccidials, a.k.a. coccidiostats, are used in poultry production to control and treat the protozoal infection, coccidiosis. This disease is caused by coccidia belonging to the genus *Eimeria*, of which *Eimeria tenella* and *Eimeria necatrix* are typical examples. The symptoms in poultry vary from diarrhoea and loss of egg production to death. Therefore, coccidiosis poses a serious economic threat to the poultry industry. A broad range of coccidiostats, of which ionophores are perhaps the most commonly used compounds, has been developed to prevent this disease. Accordingly, farmers can alternate between coccidiostats in order to reduce the risk of drug-resistant coccidia developing.

Most coccidiostats are used as feed additives to provide prophylactic control of the disease. Therefore, broilers are normally fed with coccidiostats almost throughout their lives. However, coccidiostats are typically not allowed to be

used in egg layers and therefore, eggs should, in principle, be free of coccidiostat residues. Several publications have demonstrated that accidental contamination of feeds is a major reason for the presence of coccidiostat residues in edible tissues and eggs (Cannavan and Kennedy, 2000; Cannavan *et al.*, 2000; Yakkundi *et al.*, 2002; McEvoy *et al.*, 2003). Therefore, in order to ensure food safety, it is important to address the feed industry and require regular testing of feeds.

In contrast to veterinary medicines, feed additives have not previously been associated with MRLs. However, due to the changes in the EU legislation, feed additives are now going through an MRL evaluation (Anon., 2003). Since there is a large variety of coccidiostats with no common chemical structure, a group-specific test cannot be developed for these substances. This is in contrast to antibiotics, which can be screened on a large scale on the basis of their antibacterial activity. In addition, most coccidiostats do not have a chromphore or fluorophore structure to facilitate detection.

Halofuginone

Halofuginone is a quinazolinone derivative that is used as a feed additive in the poultry industry. The withdrawal period is set at five days. There is no EU MRL for halofuginone residues in poultry, and halofuginone is not licensed in any Member State for use in layers. However, the European Medicines Agency (EMEA) has established MRLs ranging from 10 to 30 μg per kg for bovine muscle, fat, kidney and liver, which can be used as indicators of the sensitivities required from an analytical method.

The methods available for the analysis of halofuginone residues in various matrices are typically based on LC, but with expensive detection systems, lengthy extraction and clean-up procedures or high consumption of solvents (Anderson *et al.*, 1981; Tillier *et al.*, 1988; Holland *et al.*, 1995; Mortier *et al.*, 2003; Yakkundi *et al.*, 2003). Yakkundi *et al.* (2003) described a confirmatory LC–MS–MS method for poultry liver and eggs, which was validated according to EU criteria (2002/657/EC). The sample preparation consisted of an overnight trypsin digestion, liquid–liquid extraction and clean-up with SPE. Although the method worked very well, it was generally rather tedious and time-consuming, as are most confirmatory methods. Beier *et al*. (1998) reported an ELISA for the analysis of halofuginone residues in chicken liver. Also, an automated time-resolved fluoroimmunoassay, based on the concept of all-in-one dry chemistry (Lövgren *et al.*, 1996), has been developed for the screening of halofuginone residues in poultry eggs and liver (Hagren *et al.*, 2005).

Nicarbazin

Nicarbazin, an equimolar mixture of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP), belongs to the group of carbanilides. The marker residue for nicarbazin is DNC, since it is not so rapidly metabolised as HDP. Nicarbazin has coccidiocidal and coccidiostatic effects. The Joint FAO/WHO Expert Committee (JECFA) has established an MRL of 200 μg per kg for chicken liver. Nicarbazin has been on the market for decades, but recently the

use of nicarbazin, as such, was prohibited, due to incomplete toxicological data (Anon., 2001b).

Most analytical techniques for nicarbazin have been based on LC, with various detection systems (Hurlbut *et al.*, 1985; Parks, 1988; Schenck *et al.*, 1992; Draisci *et al.*, 1995; Blanchflower *et al.*, 1997a; Cannavan *et al.*, 1999; Matabudul *et al.*, 1999; Dusi *et al.*, 2000; Mortier *et al.*, 2003). A reliable confirmatory LC–electrospray MS–MS method for DNC residues in poultry eggs and liver was reported a few years ago (Yakkundi *et al.*, 2001). The method was validated according to EU criteria at concentrations of 100, 200 and 300 μg per kg in liver and 10, 30 and 100 μg per kg in eggs (Anon., 2002). Another method that is based on LC–ESI–MS, with a simple sample-extraction protocol, was described for nicarbazin in poultry feed (Cannavan *et al.*, 1999). One research group reported an elegant LC–MS–MS method for the simultaneous analysis of five coccidiostats in eggs (Mortier *et al.*, 2003). The sample was extracted with acetonitrile and, after sonication, centrifugation and evaporation steps, the sample was filtered and injected into the LC–MS–MS system. The method was validated according to Commission Decision 2002/657/EC. The CCα and CCβ for DNC were determined as 2.5 and 3.4 μg per kg, respectively.

Some workers have reported immunoassays for the screening of nicarbazin using polyclonal antibodies. Connolly *et al.* (2002) described the production of polyclonal antibodies for DNC that were raised using antigen mimics. A practical application, an automated, all-in-one dry chemistry time-resolved fluoro-immunoassay for poultry eggs and liver, utilising the above-mentioned antibodies, was published by Hagren *et al.* (2004). The concept of all-in-one dry chemistry (Lövgren *et al.,* 1996) permitted the application of a user-friendly and rapid, one-step immunoassay protocol, which is depicted in Fig. 3.2. Since all the assay-specific reagents were dispensed and dried in advance in a microtitre well, the addition of the extracted sample was the only step required to start the assay. The entire immunoassay was performed automatically by an immuno-analyser, which provided results in 18 minutes. The immunoassay was validated according to Commission Decision 2002/657/EC, which specifies the requirements for a qualitative screening assay. Although the assay was originally designed for screening purposes, quantitative results could also be obtained, if required. In addition, a surface plasmon resonance biosensor assay for nicarbazin, using polyclonal antibodies, was recently reported (McCarney *et al.*, 2003). Also, monoclonal antibodies have been generated for nicarbazin (Beier and Stanker, 2001). However, the limiting factor in the monoclonal assay seemed to be the sensitivity, which was not as good as in the above-mentioned polyclonal assays.

Nitroimidazoles

Nitroimidazoles are coccidiostat compounds that share a similar chemical structure. Their antibacterial and mutagenic activities derive from the reduction of the 5-nitro group. The reactive metabolites bind to bacterial DNA, thereby inhibiting DNA and protein synthesis in the organisms. The use of nitro-imidazoles is prohibited in the EU (Council Regulation 2377/90, Commission

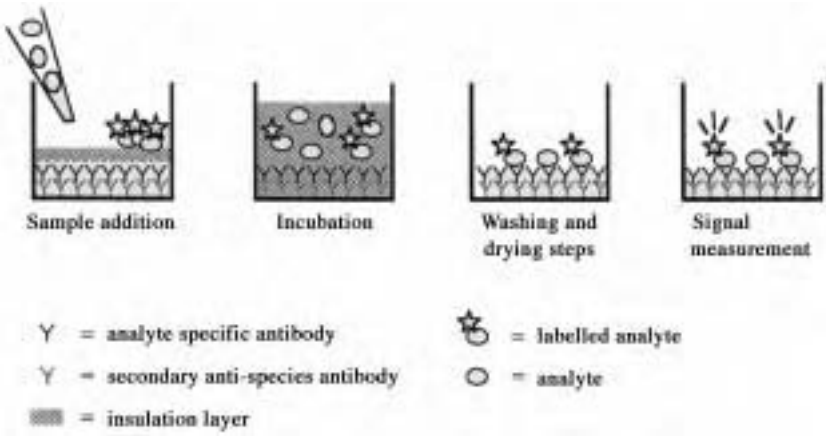

Fig. 3.2 Step-by-step presentation of the all-in-one dry chemistry assay concept used in a competitive immunoassay format.

Regulation 2205/2001), due to suspected mutagenic and carcinogenic properties. Because the identity of banned residues has to be confirmed unambiguously, MS with high specificity and identification capability is well suited to this kind of analysis.

In 1991, an LC method using UV/VIS detection was developed for multi-residue analysis of dimetridazole, ronidazole, ipronidazole and hydroxylated metabolites of dimetridazole and ronidazole, in poultry eggs, plasma and faeces, with LOQs in the range 5–10 μg per kg (Aerts *et al.*, 1991). An LC–MS method for poultry eggs, muscle and liver was reported a few years later, but only for one member of the nitroimidazole group, dimetridazole (Cannavan and Kennedy, 1997). In that study, the sample extraction was performed with toluene or dicholoromethane, and further sample clean-up was done with silica cartridges. The lowest level at which the method was validated was 5 μg per kg. Sams *et al.* (1998) described an HPLC method for the detection of dimetridazole, ronidazole and their metabolites in poultry muscle and eggs. The method could be utilised for screening by UV detection or for confirmation with MS. The method was validated at a concentration level of 5 μg per kg. Another LC–MS method for the simultaneous detection of four nitroimidazoles (dimetridazole, hydroxydimetridazole, ronidazole and metronidazole) in poultry muscle was capable of detecting residues at levels below 5 μg per kg and was described by Hurtaud-Pessel *et al*. (2000). The sample preparation included extraction with phosphate buffer and ethyl acetate, and a final washing step with hexane-carbon tetrachloride and formic acid.

Two immunoassays involving either monoclonal or polyclonal antibodies have been described for the screening of nitroimidazole residues. The monoclonal immunoassay could detect five nitroimidazoles from turkey muscle (Stanker *et al.*, 1993). However, the antibody recognised different nitroimidazoles with extremely fluctuating efficiency, and, in practice, the assay could

be used only for the detection of dimetridazole. In addition, the extraction procedure was tedious, with high solvent consumption. On the other hand, the polyclonal antibody had good cross-reactivity with metronidazole, dimetridazole, ronidazole, ipronidazole and their hydroxy metabolites (Fodey *et al.*, 2003). The IC_{50} values using standard solutions varied from 1.3 to 73.8 μg per l tested with an ELISA. Huet *et al.* (2005) reported an ELISA, using the above-mentioned polyclonal antibody, for five nitroimidazoles in eggs and chicken muscle. The CCβ values varied from 1 μg per kg to 40 μg per kg, depending on the matrix and compound. Unfortunately, an overnight incubation was included in the assay procedure, which naturally hampered the applicability of the assay.

Polyether ionophores

Ionophores are complex, high molecular weight molecules that are produced by various *Streptomyces* species. Ionophores (such as salinomycin, narasin, lasalocid, monensin, maduramicin and semduramicin) are active against Gram-positive bacteria, mycobacteria, some fungi and certain parasites and coccidia. Ionophores have a rather unspecific mode of action: they form complexes with alkaline cations and disrupt the functioning of the cell membrane, which in turn influences the osmotic pressure of the cell. The withdrawal periods for ionophores vary between three and five days. Recently, the EU has established MRLs for lasalocid in poultry tissues (Anon., 2005). Because most ionophores do not have any chromophore structures, except for lasalocid, which has an intrinsically fluorescent chromophore, they cannot be detected with HPLC, without a derivatisation step. Ionophores are normally in the form of non-volatile sodium salts and therefore are not suitable for gas chromatographic analysis as such. Consequently, most methods rely on the use of LC–MS. An illustration of a mass spectrum is presented in Fig. 3.3.

The first report of an LC electrospray MS in ionophore analysis was published by Schneider *et al.* (1991). Another method, using HPLC–electrospray MS for the detection of narasin, monensin, salinomycin and lasalocid was reported, with the advantage that only a single, quadruple MS system was needed (Harris *et al.*, 1998). By adding ammonium acetate to the HPLC mobile phase, additional diagnostic ions could be produced to further confirm the identity of the analyte. However, no results of practical applications were presented in the study. Recently, two groups reported methods based on LC–electrospray tandem MS, using chicken eggs and liver as the sample matrices. One method was able to detect simultaneously narasin, monensin and salinomycin (Rosen, 2001) and the other lasalocid, narasin, monensin and salinomycin residues (Matabudul *et al.*, 2002). The sample preparation in these studies was based on anhydrous sodium sulphate-acetonitrile extraction with SPE clean-up (Matabudul *et al.*, 2002) or methanol extraction with automated SPE clean-up (Rosen, 2001). Both methods were very sensitive and had a high throughput. Therefore, they could be used for both screening and confirmatory purposes. Matabudul *et al.* (2000) also reported a two-tier testing system for lasalocid: an HPLC with fluorescence detection for screening and an LC–MS–

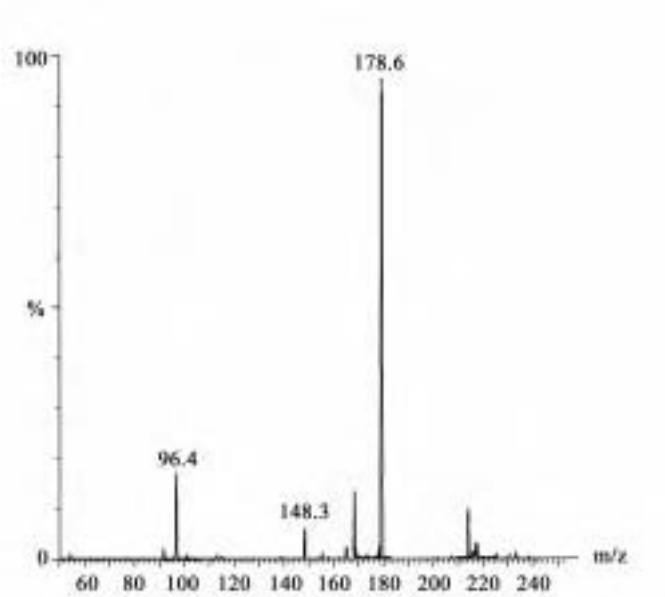

Fig. 3.3 A typical example of a mass spectrum. Each peak represents a fragment of the molecule. The intensity of the peak is proportional to the relative abundance of the fragment.

MS for confirmation. A comprehensive review of the methods available for ionophore analysis was given by Elliott *et al.* (1998).

Several enzyme immunoassays for various ionophores have been described (Kennedy *et al.*, 1995a,b, 1997; Muldoon *et al.*, 1995; Shimer *et al.*, 1996; Watanabe *et al.*, 1998, 2001). In addition, immunoassays for monensin (Crooks *et al.*, 1998b) and narasin/salinomycin (Peippo *et al.*, 2004) that rely on the use of time-resolved fluorometry have been reported. The monensin immunoassay for poultry plasma samples described by Crooks *et al.* (1998b) utilised the dry chemistry assay concept, in which all the reagents needed for the assay were dry-coated in microtitre wells. Feeding experiments were also conducted to correlate the residue concentrations in plasma with those in liver. The narasin/salinomycin assay was used to screen residues from poultry muscle and eggs. The polyclonal antibody had a cross-reactivity of 100% for narasin and salinomycin, but less than 0.1% for other ionophores. The assay was very sensitive; the LOQ was 1.8 and 0.6 μg per kg for muscle and eggs, respectively.

Triazines

Major drugs within this group are diclazuril and toltrazuril, which are derived from triazine. Diclazuril, as a veterinary medicine, is included in Annex II of Council Regulation 2377/90. For toltrazuril, MRLs have been set at 100, 200, 400, 600 μg per kg in chicken muscle, fat, kidney and liver respectively. This

substance is not permitted for use in layers. The marker residue for toltrazuril is toltrazuril sulfone, a.k.a. ponazuril. However, there are very few methods available for the analysis of toltrazuril (Benoit *et al.*, 1994; Hormazabal *et al.*, 2003). Hormazabal *et al.* (2003) reported an LC–MS method for toltrazuril and its metabolite, ponazuril, in chicken eggs and meat. An article outlining the development of an antibody for toltrazuril was published recently (Connolly *et al.*, 2003). In a buffer system, the polyclonal antibody could detect toltazuril and its metabolites at an IC_{50} value of 18 μg per l. Bearing in mind the MRLs for chicken tissues, the antibody appeared to be sensitive enough and could be applicable to toltrazuril determination in real sample matrices.

Miscellaneous
Amprolium, a vitamin B_1 analogue, and ethopabate, a substituted benzoic acid, function as coccidiostats. Ethopabate is used in combination with amprolium. However, nowadays, the use of these compounds is prohibited (Anon., 2001b). The methods for detecting these residues in chicken tissues, eggs, plasma and feed are based mainly on LC (Nagata *et al.*, 1985; Takahashi *et al.*, 1994; Tan *et al.*, 1996; Hamamoto *et al.*, 1997; Hormazabal and Yndestad, 2000; Hormazabal *et al.*, 2002; Furusawa, 2002; Yamamoto and Kondo, 2001). An HPLC–MS method for clopidol (Pang *et al.*, 2000) and an LC method with UV detection (Dusi *et al.*, 2000), capable of detecting clopidol and nicarbazin simultaneously, have been reported.

3.4 Improving control of veterinary drug residues

3.4.1 Regulatory measures

The mainstay in the food safety policies of the EU is the application of the 'farm-to-table' concept. The principles expressed in the White Paper on Food Safety by the Commission and in Regulation 178/2002, strive for a common purpose: to guarantee a high level of food safety and restore consumer confidence in the wholesomeness of food (Anon., 2000). To accomplish this, an independent European Food Safety Authority (EFSA) that provides scientific advice on various food safety issues has been established. In addition, the EFSA aims to ensure food safety within the EU and protect public health. The food safety legislation has been reviewed in order to rationalise and harmonise the legislation and bring it up-to-date. Risk analysis and the precautionary principle are applied in the assessment of food safety issues. Greater consideration is given now to the traceability of food, feed and their ingredients.

To improve residue control, the EU introduced the 'MRL Regulation', which defines the procedures for establishing MRLs for pharmacologically active compounds used in animal husbandry (Anon., 1990). Recently, Regulation 1831/2003 was issued that incorporates also feed additives into the MRL evaluation process. In addition to the EU, other authorities have set MRLs or guidelines for safe residue levels for various veterinary drugs or feed additives in

edible tissues. Since the MRLs cannot be set, or are not necessary, for all compounds, Commission Decision 2002/657/EC introduced the new concept of Minimum Required Performance Limit (MRPL) for analytical procedures. MPRLs are intended to harmonise the implementation of Directive 96/23/EC, especially in relation to those compounds for which no permitted limit has been set.

3.4.2 Analytical measures

The responsibility for residue monitoring belongs to the national surveillance scheme of each Member State (Anon., 1996). For most classes of veterinary drugs, the Member States have already adopted a cost-efficient, two-tier surveillance scheme, which consists of screening and confirmatory tests. Screening tests are regarded as high-throughput, low-cost tests, which rapidly classify a substantial number of samples as compliant or potentially non-compliant. The screening tests are biased to produce no false compliant results and only a low level of false non-compliant ones. Screening tests are typically microbiological assays or immunoassays, giving only qualitative or semi-quantitative results. The potentially non-compliant samples must be re-analysed by a confirmatory method, which is usually very specific, but also slow, high-cost and of low volume. In general, confirmatory methods are based on LC–MS techniques and provide quantitative results. In the absence of a more efficient approach, screening in conjunction with confirmation provides means to monitor veterinary drug residues in materials from food-producing animals.

Immunoassays are considered to be appropriate for screening purposes, since they are relatively rapid and easy to perform. With simple, fast and cost-efficient assays, more samples can be screened than the minimum number required by law. Consequently, a more reliable picture of the food safety status of the material will be obtained and greater protection can be provided for consumers. Immunoassays are rapidly increasing their market share and elegant detection technologies, providing highly sensitive assays, are emerging in the field of veterinary drug residue analysis. Most immunoassays for residue analysis are still based on the ELISA format. For reviews, see Beier and Stanker (1996) and Haasnoot and Schilt (2000). In addition, several applications utilising biosensors with surface plasmon resonance detection have been developed (Crooks *et al.*, 1998a; Elliott *et al.*, 1999; Baxter *et al.*, 2001; Gustavsson *et al.*, 2002; Haasnoot *et al.*, 2003b; McCarney *et al.*, 2003). Automated time-resolved fluoro-immunoassays based on the novel concept of all-in-one dry chemistry were developed recently for screening purposes in the EC Fifth Framework Programme research project ‘Poultry-check’ (http://www.utu.fi/research/residues/), as described by Hagren *et al.* (2004, 2005).

In veterinary drug analysis, the extensive sample preparation involved is generally the most time-consuming and laborious step. Consequently, automation of the entire analytical method is very difficult. However, the use of ‘predictive indicators’ might provide a solution to the automation problem.

Predictive indicators are used as markers of residue levels in various tissues. Interesting articles have appeared about the correlations between residue concentrations in bile and kidney (Crooks *et al.*, 1998a), plasma and liver (Crooks *et al.*, 1998b) and plasma and muscle (Peippo *et al.*, 2005). In addition, easy to use, dip-strip tests for liquid sample matrices have been developed (Matsumoto *et al.*, 1997; Verheijen *et al.*, 1998, 2000). Strip tests require very little sample preparation and can be used for on-site screening of the samples.

In conclusion, improvements in drug residue control must happen at many different levels. Once the legislation providing a framework for residue testing is clarified and harmonised, then the requirements of the law can be applied in a cost-effective way, with the help of novel analytical methods.

3.5 Future trends

Future developments in the field of veterinary drug analysis are likely to be comparable with those occurring in human diagnostics: rapid tests with minimal sample preparation are preferred. Therefore, it will become possible for more and more samples to be analysed at short notice. In addition, residue laboratories will need to be alert and ready to incorporate new, validated methods into their repertoires, as new drugs emerge and regulations change.

Complex sample preparation, the bottleneck of drug residue analysis, may be facilitated by automated sample pre-treatment systems. Pressurised liquid extraction may prove to be a feasible alternative method for the extraction of complex mixtures of compounds. Also, the use of predictive indicators might ease sample preparation. On-site testing of liquid samples with dip-strip tests may gain popularity. Strip tests could function as preliminary tests that are used to select potentially non-compliant samples for further analysis. For example, the use of blood samples might be an effective approach in the screening of livestock herds for residues. By performing a few random tests, an overview of the health status of the entire herd could be obtained. In the case of non-compliant results, the herd could be subjected to a more thorough testing. In addition, the testing focus might be shifted from the edible tissues to the beginning of the production chain, namely to feedstuffs, which require only minimal sample preparation prior to analysis. Accidental contamination of feed, which can be a significant source of residues in edible tissues, could be eliminated with regular quality control checks.

Rapid and user-friendly screening methods, utilising immunochemistry in new assay formats, will increase the sample throughput in routine testing. It will be interesting to see whether the screening tests will achieve better recognition in the field of veterinary diagnostics. In some cases, qualitative results from a specific screening test that can separate the analyte of interest unambiguously, might well be sufficient and then confirmation with another method would not be necessary. Multi-residue confirmatory methods, based on chromatographic and mass spectrometric instrumentation, will further improve, thereby

decreasing assay times and limits of detection. Environmental considerations will guide the analytical techniques towards reduced consumption of organic solvents. One way to accomplish this might be by means of a system called 'lab on a chip', which consists of micro-devices designed to receive only nanolitre quantities of sample on micro-chips for analysis. Techniques such as transcriptomics and proteomics may revolutionise the field of veterinary drug analysis and create totally novel concepts for detecting multiple residues in foods. Cellular mRNA or the protein profile of the cell can be analysed to produce specific 'fingerprints', which will change if cells have been exposed to drugs. Consequently, these techniques will rely on measuring the overall effect of the drug at cellular level, rather than just determining specific residue concentrations.

Since veterinary drugs will continue to play a significant role in animal husbandry, novel methods are needed for residue control. The development and validation of sophisticated analytical methods will require investments, both scientific and economic, before cost-efficient monitoring schemes based on the new techniques can be applied routinely. However, such an approach will be worthwhile, if a high level of food safety and consumer protection can be guaranteed in the future.

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4

Modelling risks from antibiotic and other residues in poultry and eggs

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4.1 Introduction

There is extensive public concern about chemical contamination of edible animal products (meat, milk and eggs), including poultry meat (Shull and Cheeke, 1983; National Research Council, 1999; Paige *et al.*, 1999; Resurreccion and Galvez, 1999). Some of these are justifiable concerns but, in many cases, apprehension about chemical residues is not based on scientific information. In a recent consumer survey, Resurreccion and Galvez (1999) reported that more than 70% of respondents considered animal drug residues or pesticides in meats to be an extreme health concern (Fig. 4.1). In the same study, 77% of consumers believed that bacterial pathogens on meats were also an extreme health concern. Thus, in the public's perception, drug residues and pesticide contamination are considered as important as foodborne pathogens to the health and safety of those purchasing retail meats. In most countries, there are numerous regulatory requirements and restriction and enforcement activities to ensure the absence of toxic levels of chemical residues in the food supply. In the case of foodborne pathogens, even with extensive scientific research, it is impossible at present to eliminate all bacterial pathogens from foods. As scientists, we must recognize the public's perceptions and strive continually to provide sound scientific data to educate both consumers and officials about the safety of the food supply. This review will focus on modelling residues in edible poultry tissues and the unique issues associated with residue assessment for eggs.

Poultry can be exposed to chemicals in two basic ways, either via intentional or unintentional routes. Intentional exposure occurs when the birds are treated with compounds, such as antibiotics and anticocciodostats, for the prevention or

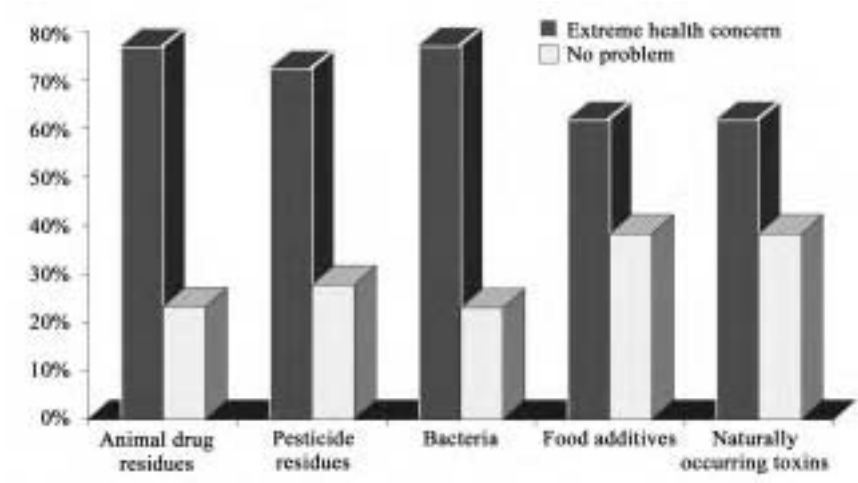

Fig. 4.1 Consumer survey: concern about potential food contamination in meat (reprinted with permission from *Food Technology* (Resurreccion and Galvez, 1999)).

treatment of disease, or to enhance bird growth and feed efficiency. Unintentional exposure involves pesticides, dioxins, heavy metals, etc. Some of these chemicals are man-made, e.g. most pesticides, whereas others are naturally occurring, e.g. heavy metals. In many cases, exposure of poultry to these chemicals leads to their incorporation, either directly or as metabolites, into the edible tissues (meat and adhering fat, eggs, kidney and liver). However, it must be emphasized that the mere occurrence of residues does not mean that the food is necessarily unsafe.

Throughout the world, many chemical products are used in the production of poultry. In most developed countries, there are governmental regulatory requirements for the approval and safe use of these products. For example, in the USA, the United States Food and Drug Administration (US-FDA) is responsible for the approval of many chemical products used in animal production, e.g. animal drugs (Federal Food, Drug, and Cosmetic Act, 1958). After approval, the US-FDA's sister agency, the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS), in cooperation with the US-FDA, monitors the food supply, not only for approved products, but also for unintended contaminants, such as pesticides and dioxins. Many other countries follow similar regulatory procedures (Botsoglou and Fletouris, 2001).

4.2 Mechanism of transfer

The ability of a chemical to produce residues in edible tissues depends upon the pharmacokinetic principles of absorption, distribution, metabolism and

excretion. Because of the large-scale nature of many poultry operations, the most economical and frequent route for intentional dosing of poultry is either via the feed or drinking water (National Research Council, 1999). Unintentional exposure also occurs through these routes (Mussman, 1975; Shull and Cheeke, 1983; Lovell *et al.*, 1996; Hume, 2002; Schafer and Kegley, 2002).

The efficacy of many of the chemicals used is dependent upon efficient absorption. For example, many animal drugs, such as antibiotics, were selected because of their efficient absorption and distribution to target tissues for therapeutic purposes. Pesticides were also selected for efficient transfer into targeted biological species. In the case of some man-made chemicals, such as polychlorinated biphenyls (PCBs), absorption into living organisms is an unintentional property of these substances. Whether intentional or unintentional, the ability of the chemicals to be absorbed by poultry may have the undesirable consequence of causing residues in edible tissues.

If a chemical is absorbed by poultry, it is likely to be distributed within the body of the bird. Depending on its properties, e.g. size, pK_a, the chemical may be deposited preferentially in selected, edible tissues. For example, many antimicrobials are deposited preferentially in either egg yolk or albumen (Kan *et al.*, 1998; Zurhelle *et al.*, 2000; Roudaut and Garnier, 2002). Other chemicals are found mainly in areas such as adhering fat, kidney or liver (Eisele *et al.*, 1985; Pang *et al.*, 2001; Schafer and Kegley, 2002). At present, it is impossible to predict from its chemical properties, the full potential of a substance to accumulate as a residue in animal tissues, without comprehensive pharmacokinetic studies (Baynes *et al.*, 1999; Kan and Petz, 2000; Gehring *et al.*, 2004). Therefore, when a chemical is intended for deliberate use in poultry, the pattern of residue incorporation is determined as a requirement of regulatory approval, to ensure the safety of the edible tissues for consumers (see Section 4.3).

Following absorption, a chemical may be excreted either unchanged or converted by enzymatic processes to a substance that is more readily excreted from the body. This process usually takes place in the liver. As a consequence of such metabolism, the original chemical compound may be converted to an inactive metabolite, modified from an inactive to an active form or converted to a substance with different activity (*Merck Veterinary Manual*, 1998). For example, when chickens are dosed with the fluoroquinolone, enrofloxacin, some of the antibiotic is metabolized into another active fluoroquinolone, ciprofloxacin (Anadon *et al.*, 1995; Knoll *et al.*, 1999; Donoghue and Schneider, 2003). Therefore, when evaluating residue transfer into edible tissues, it is helpful to know the pattern of metabolism, so that methods can be developed to detect any biologically-active residues that could occur in edible tissues.

It is not within the scope of this chapter to present a comprehensive description of the pharmacokinetic principles associated with the metabolism of ingested chemicals. There are many excellent reviews and book chapters that describe the relevant principles in detail, e.g. *Merck Veterinary Manual* (1998).

4.3 Establishment of safety tolerances for residues

There are two major aspects to evaluating the safety of residues in foods. These are determining the toxicity and quantity of the chemical residue in the edible product. For intentional application of chemicals in poultry, most developed countries require safety testing prior to use (Botsoglou and Fletouris, 2001). Toxicity studies are conducted to determine the acute and chronic toxicity of the chemical compound and any potential metabolites. Toxicity can vary widely between compounds. For example, the tetracycline antibiotics are used extensively in poultry, whereas chloramphenicol is prohibited for animal use in many countries, because of its potentially life-threatening toxicity (Botsoglou and Fletouris, 2001).

Acute toxicology studies evaluate effects such as allergic reactions, whereas long-term chronic studies identify other possible problems, such as development of cancer. The dose of a particular compound that produces no adverse effect in either acute or chronic long-term studies, known as the 'no observable effect level' (NOEL), is determined and used to calculate a tissue-tolerance concentration. The tolerance is a safe concentration of the chemical in edible tissues. In the USA, the US-FDA establishes tolerances by taking the NOEL, reducing that concentration by a safety factor of 100 or 1000-fold, and multiplying the amount by the average daily intake (ADI) of a 60 kg human adult (see www.fda.gov/cvm/guidance/published.htm). The 100-fold safety factor is used for chemicals with a long history of safe use, e.g. tetracyclines, whereas a 1000-fold safety factor is applied to newer chemical compounds. The tolerance is also considered the maximum residue limit allowed in foods.

Once the tolerance is established, pharmacokinetic studies can be conducted to ensure that the residues in tissues do not exceed the tolerance levels in meat or eggs being sold to consumers. Usually, uptake and depletion studies are performed in poultry for the chemical in question, radiolabelled with C^{14}. After dosing, edible tissues are evaluated at different times to determine the points at which total residue concentrations decrease below the established tolerance. An example of a theoretical dosing study for an antibiotic is depicted in Fig. 4.2. Assuming the tolerance was set at 0.3 ppm during the toxicity phase of testing (see previous paragraph), then, for this example, the edible tissues would be safe for human consumption two days after withdrawal of the antibiotic. This two-day withdrawal period would be specified on the product label. These types of study also identify the 'marker' residue, which is either the parent chemical or its metabolite, and represents the total concentration of the chemical product in the edible tissues. The marker residue is used to develop analytical methods for use in monitoring retail poultry meat and eggs to ensure that residues do not exceed tolerance levels. (Freidlander *et al.*, 1999; Donoghue, 2003).

The description of safety testing given in this section uses the example of the US regulatory standards. Many other countries follow variations of these procedures. In addition, international efforts are being made to standardize the regulatory safety and approval process for all relevant chemicals used in animal

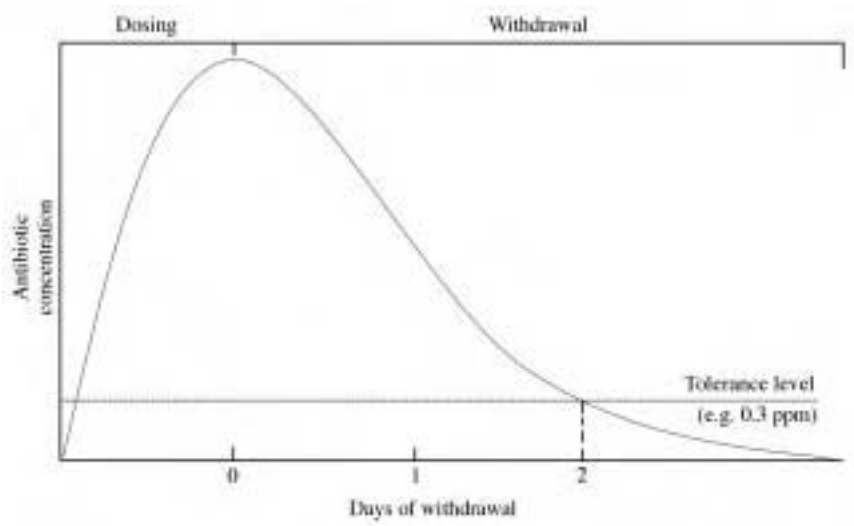

Fig. 4.2 Theoretical depletion of antibiotic residues in edible poultry tissue (reprinted with permission from *Poultry Science* (Donoghue, 2003)).

production. An excellent review of different national standards for regulatory safety testing is given by Botsoglou and Fletouris (2001).

The scientific approach described above is extremely effective for ensuring the safe use of various chemicals in the efficient production of healthy poultry flocks. Unfortunately, poultry are also exposed to chemicals that have not undergone such rigorous safety testing. In some cases, there is off-label or illegal use of chemicals, or the potential for unintentional exposure to substances such as pesticides in the feed. These situations can provide different challenges to regulatory authorities that are trying to ensure the safety of food. Decisions that affect food safety are sometimes made on the basis of limited scientific knowledge. In all cases, the best decisions are reached only when the tolerance and tissue residue patterns are known. At present, it is impossible to predict dependably and accurately the toxicity or residue concentrations in animals, purely on the basis of the properties of the chemical in question (Baynes *et al.*, 1999; Kan and Petz, 2000; Gehring *et al.*, 2004). Usually, however, at least some limited scientific information is available on the toxicity or kinetics of residue incorporation, and this information can be used to model the pattern of residue transfer.

4.4 Modelling and predicting levels of residue transfer

As stated above, the most through assessment of the safety of chemical residues in poultry is accomplished by toxicology testing and establishing the maximum allowable residue limit (tolerance) for a specific chemical at a specified dose.

Unfortunately, in some circumstances, these data are not available. In those cases, modelling the residue profile may be required.

The ability to model residue concentrations and the length of time residues persist in edible tissues are invaluable to veterinarians, poultry producers and government regulatory authorities. Modelling can predict potential problems and direct veterinarians and producers to the most effective means of dosing to prevent violative residues. Modelling can also assist regulatory authorities by predicting the levels of chemical exposure that are most likely to create problem residues, so that analytical resources can be directed at the key chemicals, thus ensuring proper enforcement.

Modelling the transfer of chemical residues into edible poultry tissues is a form of risk evaluation. Risk associated with residues depends upon the toxicity of the particular chemical and the amount occurring in the tissues. Because toxicology data are available for a wide range of chemicals (Renwick, 2004; Wexler, 2004), the limiting factor in risk evaluation is often knowledge of the residue concentrations in the edible tissues.

4.4.1 Modelling residue transfer into edible poultry tissues other than eggs

Pharmacokinetic residue models have been developed for specific chemicals and a number of different situations. The first is where extensive information is available on residue concentrations, but not for the dose, route of administration, species, etc. This situation can occur in off-label or illegal usage. In the USA, for example, licensed veterinarians have the authority to treat poultry with most drugs, regardless of the approved, product-label directions. They can dose poultry with higher concentrations of a drug than those recommended, or even use a drug that is not approved for poultry. Because of their medical expertise, it is assumed that they will not dose the birds in a manner that creates residues or residues exceeding an established tissue tolerance. The law that provides this authority is the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA; Paige, *et al.*, 1999). Also contained in the law is a list of chemical compounds, e.g. diethylstilbestrol, that cannot be used in food-producing animals, even by licensed veterinarians.

In most cases, licensed veterinarians do not have extensive experience in conducting pharmacokinetic studies and evaluating residue transfer into edible tissues. Therefore, the possibility exists that mistakes will be made and unsafe concentrations of residues will occur. In an effort to provide veterinarians and poultry producers with dosing information that would avoid unsafe residues in animal tissues, a USDA-backed group of veterinarians and pharmacokineticists has established the Food Animal Residue Avoidance Database (FARAD; Payne *et al.*, 1999). This group can respond quickly to requests for dosing recommendations via the telephone or their website (www.farad.org; see description below).

Illegal dosing of poultry can also create unsafe residues in foods. For example, the use of chloramphenicol is illegal for food animals in the USA,

because of the potential to create life-threatening blood dyscrasia (National Research Council, 1999). Prohibited compounds are banned by regulatory authorities on the basis of extensive scientific data, indicating that they are both toxic and residue-forming in foods. Since there is considerable pharmacokinetic information already available for these chemicals, there is greater potential for more accurate modelling.

A second application of modelling is in relation to exposure of animals to chemicals for which there is a paucity of residue incorporation data. This makes it much more difficult to model residue transfer accurately and the situation can arise where poultry are exposed to chemicals that are not intended for use in animal production. Examples include pesticides, PCBs and dioxins. Potentially, there are many man-made chemicals that can occur in the food supply (Mussman, 1975; Kan, 1978; Headrick, *et al.*, 1999; Hume, 2002; Schafer and Kegley, 2002). Usually, such chemicals were never intended by the manufacturer for use in food animals and therefore were not evaluated for residue transfer into edible tissues. It is not economically feasible for scientists to evaluate all of these chemicals. In poultry, one of the most common vectors of contamination is the feed. A study by US-FDA scientists found that 457 of 545 samples of mixed feed used for domestic animals, including poultry, contained detectable amounts of various pesticides (Lovell *et al.*, 1996). Recently, there has been extensive concern about dioxin contamination of animals. Dioxins are considered to be among the most toxic chemical compounds in existence (Hume, 2002). The majority of dioxins are believed to be man-made, but some may be produced naturally. Poultry and other food animals were contaminated with apparently naturally-occurring dioxins, when the anti-caking agent, ball clay, was used in feed in the USA (Rappe *et al.*, 1998; Headrick, *et al.*, 1999; Hume, 2002). In 1998 and 1999, dioxin-contaminated feed was unknowingly fed to Belgian animals (Covaci *et al.*, 2002; Bernard and Fierens, 2002), resulting in the condemnation of many poultry products. The concern about Belgian foods was so great that there were international recalls and a ban on the importation of animal products from Belgium (Hume, 2002).

There are ongoing efforts to model the quantity and duration of chemical residues in poultry tissues, including meat and adhering fat, liver and kidney. Numerous studies have been carried out on residue modelling (Concordet and Toutain, 1997; Martin-Jimenez and Riviere, 1998; Baynes *et al.*, 1999; Fisch, 2000; Gehring *et al.*, 2004). In 1982, the USDA, in an effort to enhance scientific expertise on residues and promote science-based decision-making, supported the development of FARAD (Payne *et al.*, 1999). This is a consortium of scientists from the Universities of California, Florida and North Carolina State, with expertise in chemical-residue incursion of animal tissues. The group developed a modelling program called the Extrapolated Withdrawal-Interval Estimator (EWE). The EWE program estimates the toxicity and quantities of a specific chemical residue in edible tissues (Martin-Jimenez *et al.*, 2002). Once poultry is no longer exposed to the chemical compound (withdrawal period), it is possible to estimate if or when residues in the edible tissues are reduced to

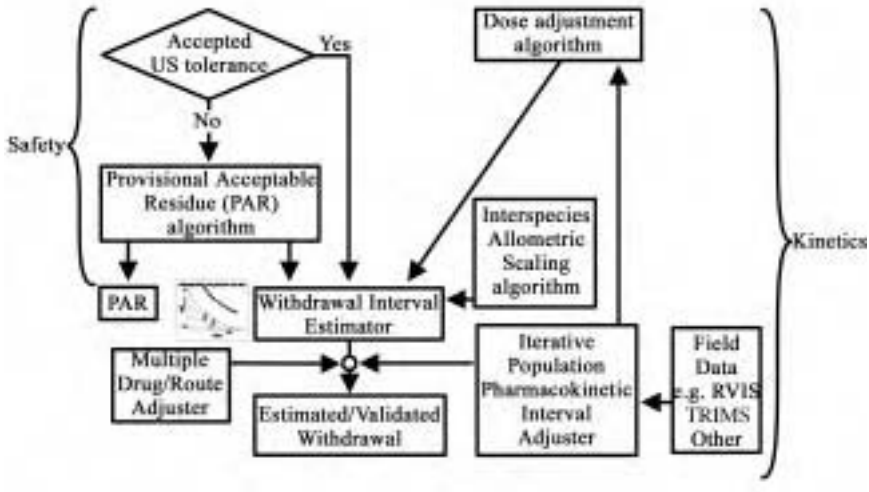

Fig. 4.3 The Extrapolated Withdrawal-Interval Estimator (EWE) Residue Model from the Food Animal Residue Avoidance Databank (FARAD) website (www.farad.org) (reprinted with permission from the FARAD (2004)).

acceptable, safe concentrations (Fig. 4.3). The EWE program utilizes available pharmacokinetic data, historical experience, foreign drug approvals, interspecies extrapolations and extrapolations from approved products to provide estimates for specific chemicals. This program is updated and refined as new residue data become available. Data for these updates come from research carried out by other scientists, government surveillance activities, etc., as well as from information generated by FARAD scientists themselves. Some recent examples of the research generated by FARAD to refine the EWE program include Baynes *et al.* (1999), Craigmill and Cortright (2002), Martin-Jimenez *et al.* (2002) and Gehring *et al.* (2004). The FARAD group has also compiled an extensive literature review on residues associated with many veterinary antimicrobials and therapeutic drugs. It is contained in three books entitled *Handbook of Comparative Pharmacokinetics and Residues of Veterinary Therapeutic Drugs* (Craigmill *et al.*, 1994); *Handbook of Comparative Veterinary Pharmacokinetics and Residues of Pesticides and Environmental Contaminants* (Sundlof *et al.*, 1995); and *Handbook of Comparative Pharmacokinetics and Residues of Veterinary Antimicrobials* (Sundlof *et al.*, 1996).

The kinetics of residue transfer into most edible poultry tissues follows the traditional pharmacokinetic principles of absorption, distribution, metabolism and excretion. There is a dynamic relationship between chemical transfer into and out of tissues. Once animals are no longer exposed to a chemical, it often continues to be excreted from the body, and tissue concentrations are reduced accordingly. The length of time needed for 50% of the original concentration to disappear from an edible tissue is referred to as the 'elimination half-life' (Gehring *et al.*, 2004). For example, after 1, 3, 5 or 10 elimination half-lives,

there would be respectively 50, 88, 97 or 99.9% less residue in the tissues. For some chemical compounds, the elimination half-life is already known, and veterinarians commonly assume that, after five half-lives, residue concentrations in the tissues will be below any regulatory tolerance levels (Gehring *et al.*, 2004). Although these pharmacokinetic principles are useful in model development for most tissues, the same relationships do not adequately depict residue incorporation into eggs.

4.4.2 Modelling residue transfer into eggs

Modelling residue transfer into eggs offers unique challenges. It appears that, unlike most tissues, residue transfer in eggs occurs in only one direction. Instead of an exchange of chemicals into and out of eggs, chemicals are only transferred into eggs (Donoghue *et al.*, 1996; Donoghue and Myers, 2000). This unusual situation is due to the intended biological purpose of eggs and the unique dynamics of egg formation. Although eggs are thought of as food, the egg is actually the reproductive unit of the hen. It is formed with the intention of being discharged from her body for incubation and hatching. Therefore, from a chemical residue perspective, it acts more like an excretory product. Unlike other such products, e.g. urine and faeces, developing egg yolks will take days to weeks to be excreted (Johnson, 1999). It is therefore necessary to understand the dynamics of egg formation before attempting to model residue transfer into eggs.

The edible components of the egg, the albumen and yolk, are formed in two distinct ways. The albumen proteins are synthesized and usually secreted daily (except for skip days; Johnson, 1999). Yolks are formed in the ovary in three different stages. These are the small white, small yellow and large yellow yolk phases. Within the ovary, there are thousands of small white follicles that, in most cases, remain dormant and never grow into a mature yolk. Once the hen reaches reproductive age, some of the small white yolks will be selected and grow into small yellow ones (Fig. 4.4). The latter will be retained in the hen's ovary for up to about six weeks, until recruitment and growth into a large yellow yolk. Most yolk formation occurs during this time. These large yellow yolks will steadily accumulate yolk material, growing from about 0.2 g to 15 g within a two-week period (Fig. 4.4; Johnson, 1999). Following completion of growth, the yolk will be released from the ovary into the reproductive tract, and albumen, water and minerals, plus an eggshell, will be added. Then, the completed egg will be ovipositioned. The recruitment of small white and small-to-large yellow yolks continues for the hen's entire egg-laying cycle.

Researchers have speculated on the contribution of residue accumulation and storage in the developing yolk to the total residue content of whole eggs (Geertsma *et al.*, 1987; Roudaut *et al.*, 1989; Donoghue *et al.*, 1994). However, when conducting traditional dosing trials, it is impossible to distinguish between the two. This is because, even after withdrawal of the chemical, there may still be residues in other body compartments, e.g. blood, that are transported into

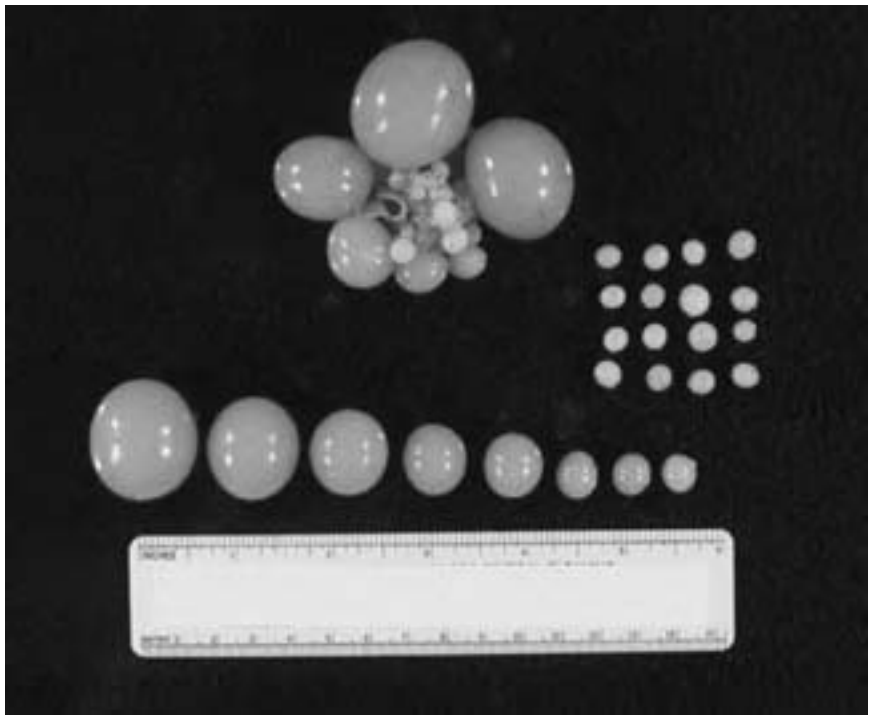

Fig. 4.4 Photograph of an intact hen ovary (upper centre) and dissected yolks from the large (lower) or small (upper right) phase of yellow yolk formation. Large yellow yolks (>0.2 g) are within two weeks of ovulation and arranged within a follicular hierarchy. The largest, heaviest yolk usually ovulates within 24 hours; the others subsequently at 24 hour intervals, in order of size. Small pre-ovulatory yolks (<0.2 g) are within 2–6 weeks of ovulation (reprinted with permission from the *Journal of Agricultural and Food Chemistry* (Donoghue and Myers, 2000)).

developing yolks and subsequently whole eggs. In an effort to resolve this issue, hens were dosed with either antibiotics (Donoghue *et al.*, 1996) or a pesticide (Donoghue *et al.*, 1997b), euthanized 24 hours after dosing, and the residue content of the developing yolks determined. Because the hens were euthanized, it would not be possible for any additional residue transfer to occur after the dosing period. Even following an exposure period of only 24 hours, developing yolks, days to weeks from ovulation, had stored substantial quantities of chemical residues. It is apparent, therefore, that residues can be stored, for subsequent incorporation into whole eggs, for long periods after drug exposure. To further test this possibility, hens were dosed with the short half-life and rapidly-excreted antibiotic, ampicillin (half-life approximately 90 min; Zurich *et al.*, 1984). Ampicillin residues were detected in whole eggs for up to seven days after only a single dose (Donoghue *et al.*, 1997a). It is doubtful whether ampicillin residues persisted in other body compartments and transferred into yolks during drug withdrawal, because of the short half-life and the fact that

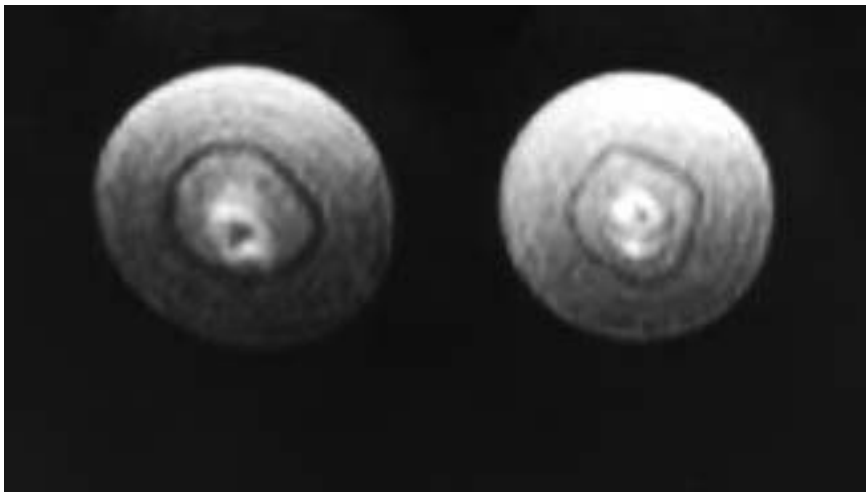

Fig. 4.5 Eggs collected from two different hens days after a single injection of magnevist. Notice a single dark ring of incorporated residue (reprinted with permission from the *Journal of Agricultural and Food Chemistry* (Donoghue and Myers, 2000)).

plasma ampicillin was undetectable 24 hours after the single dose. In an additional study, hens were dosed with the ultra-short half-life contrast agent, magnevist, and residues were observed in the yolks of whole eggs, using magnetic resonance imaging, for up to six days after a single dose (Fig. 4.5; Donoghue and Myers, 2000). Therefore, it is likely that the residues were incorporated into the developing yolks during dosing and may occur in whole eggs for a number of days after drug withdrawal.

A surprising finding of these studies was that the pattern of uptake of residues was similar for different classes of chemicals in developing, large yellow yolks (Donoghue *et al.*, 1996, 1997b), and this was confirmed with two different classes of antibiotic (β-lactam-type and tetracycline) and an organochlorine pesticide (Donoghue *et al.*, 1997b). There is a physiological basis for this residue pattern. Residues are incorporated into yolks in the same manner as the deposition of yolk material (Donoghue *et al.*, 1996). Therefore, on the same basis, it was possible to develop a model predicting the pattern of residue incorporation into whole eggs (Donoghue *et al.*, 1996, 1997b; Donoghue, 2001). Because the temporal relationship between developing large yolks is known (Johnson, 1999), it is possible to predict the relative residue concentrations in the yolks of whole eggs. The model can be used by regulatory authorities to estimate the number of days that eggs will contain residues from hens dosed with a diverse range of chemicals.

There are some limitations to the model. The model predicts the pattern of residues in eggs laid subsequently by dosed hens, but not the quantities in specific eggs. Although the pattern of residue transfer appears to be consistent for different chemicals, the quantities of residues will vary (Donoghue *et al.*,

1996, 1997b, Donoghue, 2001). Thus, it is necessary to have an idea of the amount of residue incorporated for each specific chemical. Fortunately, the residue concentrations are known for a number of chemicals that may be used in egg production (Kan, 1978; Geertsma *et al.*, 1987; Roudaut *et al.*, 1987; Roudaut and Moretain, 1990; Kan *et al.*, 1998; Kan and Petz, 2000; Zurhelle *et al.*, 2000; Donoghue *et al.*, 1994; Donoghue and Hairston, 1999, 2000; Shaikh *et al.*, 1999; Pensabene *et al.*, 2000; Schenck and Donoghue 2000; Lehotay *et al.*, 2001; Donoghue and Schneider, 2003; Elkin *et al.*, 2003; Schneider and Donoghue, 2004). Furthermore, if the residue content of just one egg in the sequence is determined, then the model can predict the likely amount of residue for the entire sequence of eggs, following exposure. Another limitation of the model is that it is more accurate for chemicals with a short half-life, e.g. many antibiotics. For chemicals with longer half-lives, such as some pesticides, it is necessary to take account of the amount transferred into developing yolks, not only during the dosing period, but also after withdrawal of the chemical (Donoghue *et al.*, 1997b; Donoghue, 2001). Thus, even after exposure has ended, long half-life chemicals will persist in the body and may continue to transfer to the developing yolks and subsequently whole eggs. Despite the limitations, the model will help poultry producers and veterinarians to make informed decisions about limiting the transfer of residues into eggs.

Other efforts have been made to predict preferential deposition of a chemical into either albumen or yolk, depending on the properties of the substance. Yolk and albumen are very different in chemical composition. Yolk consists of 48% water, 32.5% fat, 17.5% protein, 1.0% minerals and 1.0% carbohydrate, whereas albumen is primarily water and protein (84, 11, 1.0 and 0.8%, respectively, of water, protein, carbohydrate and minerals; North, 1984). Because of these differences in chemical composition, it is known that many extraneous chemicals are preferentially deposited into one or other of these edible compartments. Such information could benefit regulatory decision-making on egg safety. Many eggs are separated into albumen and yolk for independent use in further-processed foods, e.g. ice cream, angel food cake. The ability to predict preferential deposition of particular residues into either food matrix would allow a more accurate assessment of the safety of these materials.

Extensive studies were conducted, and reviewed by Kan and Petz (2000), to evaluate preferential deposition of chemicals into albumen or yolk. As reported by these authors, the dosing of hens with 11 different sulfonamides led to a differing distribution between the two egg compartments that could not be explained on the basis of the pKa values or lipid-solubility characteristics of these antibiotics. Despite the large database on this topic, it was concluded that there were still no clues as to the properties that determine the observed distribution of a particular chemical (Kan and Petz, 2000). Thus, at present, the best predictor of residue-transfer into eggs is an understanding of the pattern of incorporation into developing yolk and the amounts that accumulate.

4.5 Implications for effective control

The procedures described in this chapter provide a framework for evaluating the safety of chemical residues that occur in edible poultry tissues. However, ensuring the safety of the food supply depends on proper enforcement of the key principles. Without a strong surveillance and compliance effort, either intentional, illegal or inadvertent exposure to chemicals may produce harmful residues in the food supply. Many countries have active regulatory supervision to ensure compliance and to detect and eliminate harmful residues (Botsoglou and Fletouris, 2001). For example, in the USA, the US-FDA, the USDA-FSIS and the Environmental Protection Agency (EPA) all collaborate actively in an effort to prevent the occurrence of harmful residues. The USDA-FSIS has a national residue-monitoring programme that targets specific residues in edible animal tissues, including those of poultry (Paige *et al.*, 1999). This programme monitors not only foods produced in the USA, but also those imported from elsewhere.

In the past, there was no deliberate intention to produce foods containing harmful residues. Even the illegal application of chemicals in poultry production was done for economic benefit and not with malicious intent. Recent bioterrorist activities may change this situation. Both pre-harvest and post-harvest food monitoring will need to be implemented to counteract this type of threat. Fortunately, because of the wide distribution of food production and processing operations in most countries, it would be difficult to contaminant a significant amount of the food supply at any one time. Furthermore, many chemicals that may be used deliberately to contaminate growing poultry would also make the birds ill and thus serve as an early warning signal. The unintentional contamination of poultry with dioxin in Belgium was discovered because the birds became ill (Hume, 2002). However, although the number of affected poultry was relatively small, the product recalls and international ban on selected Belgian animal products had a significant economic impact. Therefore, it is necessary to develop and maintain a strong government regulatory monitoring system to detect and limit the food safety problems and subsequent economic impact.

4.6 Future trends

There will be a continued need to model residue transfer into edible poultry tissues, as new chemicals are developed. Moreover, there will probably be additional regulatory supervision of the food supply with regard to chemical use and contamination. Recent concerns about the development of antibiotic-resistant pathogens in animals, following antibiotic treatment, and the potential for bioterrorism have focused more attention on the safety of the food supply. In the European Union and USA, restrictions have been proposed or instituted on the use of some antimicrobials in food animals, including poultry (Hardy, 2002; McDermott *et al.*, 2002; http://www.fda.gov/cvm/antimicrobial/

FQWithdrawal.html). Modelling the persistence of antibiotic residues in non-edible gut tissues and their interactions with enteric microflora will assist the appropriate usage of antimicrobials and limit development of antibiotic-resistant, human pathogens.

The threat of bioterrorism also poses new and difficult challenges to regulatory authorities. In most cases, government enforcement policies have been developed on the premise that any contamination of the food supply is an inadvertent act. Because of budget restrictions, however, most food production facilities are self-governed, with only limited regulatory monitoring. With the increased potential for terrorist acts, governments and food producers will need to increase their vigilance. In fact, the US-FDA recently released a new regulatory policy to enhance the security of the food supply in relation to bioterrorism concerns (http://www.fda.gov/OHRMS/DOCKETS/98fr/04-12366.pdf). Because of these issues, residue modelling will need to be developed for non-traditional applications. Exotic chemicals, not normally associated with food animals and their contamination, may have to be modelled to predict the potential dangers from terrorist acts. Thus, model development and use may play an enhanced role in the future in protecting the safety of the food supply.

4.7 Sources of further information and advice

There are a number of government agencies and private organizations that provide scientific and policy updates on efforts to restrict the unsafe accumulation of chemical residues in foods. These organizations include, for example, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority, the US-FDA, the USDA-FSIS, FARAD, the US-EPA, the American Feed Industry Association, Food Standards Australia-New Zealand. Many of these organizations provide extensive scientific data and information on regulatory policies on their websites or via published articles. Additional information can be obtained using internet-based, scientific search programs, such as Pubmed®, Agricola®, Medline® and Toxline®.

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5

Pathogen populations on poultry farms

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5.1 Introduction

The objective of this chapter is to describe in detail the distribution and behaviour of the major foodborne pathogens in poultry production. Therefore, the chapter will focus mainly on *Salmonella* and *Campylobacter*, the two pathogens for which poultry products are known to be a significant source. Consideration will be given to the prevalence of these organisms in different types of poultry flock in relation to the control measures used. However, the presence of an organism in a particular part of the poultry production pyramid does not necessarily create a significant risk, e.g. *Campylobacter* in egg production or breeding flocks, non-invasive *Salmonella* serovars in egg production, so the relationship between the organism and production sector will also be considered. A good understanding of possible sources of infection is vital to effective control, so the major sources, such as feed, hatchery, vertical transmission, persistent farm infection and environmental contamination, will be discussed. Once the organisms are introduced onto the farm, it is important to understand their likely behaviour with regard to direct and indirect spread, involvement of farm pests, contamination of equipment and personnel, airborne spread and survival in environmental niches. Only then can the organisms be effectively controlled.

There are major differences in the behaviour of *Salmonella* and *Campylobacter* in the environment, which present challenges to poultry companies and incur considerable costs for monitoring and control. Effective monitoring is very important, both for understanding pathogen behaviour and ensuring its control. Sampling methods are therefore fundamental and will be discussed in detail, while methods of pathogen detection are considered in Chapter 20. Equally

important is the application of intervention measures and knowledge of their limitations. This applies to both statutory controls, which are currently in place for *Salmonella*, and additional, voluntary controls for this organism and others. Since the epidemiology of the two main organisms is complex, it is necessary to use a battery of methods to ensure that control is maintained. These include biosecurity, hygiene measures and, for *Salmonella*, vaccination, feed additives and competitive exclusion, which are applied to different extents in the various sectors of the industry. Future developments in terms of improved vaccines, nutritional approaches and genetic resistance to infection may further improve control of these pathogens, but many measures are already available, and are potentially effective, as long as they are applied correctly. The common failure to fully implement available control measures results from an unfavourable cost-benefit ratio in most of the poultry producing countries.

5.2 Foodborne pathogens in poultry and their significance

Modern food production is so complex that full control of pathogens at every stage is very difficult, since not all components of the supply chain may be under close, central control (Sanders, 1999). Every time there is a change in the way the food chain operates, a new selective pressure may be created for novel or previously insignificant pathogens (Miller *et al.*, 1998). Organisms are responding to environmental challenges the whole time and new forms, such as *Escherichia coli* O157, *S.*Enteritidis PT4, 8 and related egg-invasive strains (WHO, 1989; Haeghebaert *et al.*, 1998; FAO/WHO 2003), as well as the *S.*Typhimurium DT104 complex, are recent examples of this (Miller *et al.*, 1998; Rabsch *et al.*, 2001). There is a large range of potential pathogens that may be present in commercial poultry and therefore may appear, theoretically, on poultry meat or eggs (EC, 2000). This list also includes commensal organisms with antimicrobial resistance, such as *E.coli* or *Enterococcus faecium* that may, in some circumstances, act as opportunist pathogens or as a potential reservoir of resistance genes, such as *VanA* in *Ent. faecium* or plasmid-mediated trimethoprim/sulphonamide resistance in *E. coli*. Environmental spread of pathogens and resistant organisms originating from poultry waste is also important, particularly when fresh salad vegetables or water supplies become contaminated (Meng and Doyle, 2002). Although the range of poultry-associated pathogens is large, the most significant organisms in the present context are *Salmonella* and *Campylobacter* (Thorns, 2000). These are responsible throughout the world for 90% of identifiable, bacterial zoonoses that are foodborne.

5.2.1 *Salmonella*

Salmonella is an important foodborne pathogen worldwide, because of the frequency of its occurrence and its potential pathogenicity (Barrow, 2000; Meng and Doyle, 2002). An important feature of the organism is the epidemic spread

of certain clones, e.g. *S.*Enteritidis PT4 or *S.*Typhimurium DT104 (Threlfall *et al.*, 1993; Low *et al.*, 1997). *Salmonella* survives well in the environment and has been involved in large outbreaks of foodborne illness where vegetables, fruit and spices have been contaminated by animal or human faecal waste or contaminated water (Bryan and Doyle, 1995; EC, 2000). It can also cause long-term contamination of industrial facilities, such as incubators for hatching eggs, feedmills and poultry processing plants. A major concern with *Salmonella* is the decrease in susceptibility to newer antimicrobials, such as fluoroquinolones and extended-spectrum cephalosporins (Rabsch *et al.*, 2001). In recent years, a type of *S.*Newport with multiple resistance has emerged and spread widely in the USA, as has *S.*Paratyphi B var. Java, which also has multiple resistance, in some European countries. It is important that the international spread of such strains is limited as far as possible.

5.2.2 *Campylobacter*

This organism, especially *C. jejuni*, is the leading cause of foodborne zoonoses in most developed countries (Harris *et al.*, 1986; WHO, 2001). It is widespread in poultry production (Evans 1992; Bryan and Doyle, 1995), and poultry is often implicated in case-control studies (Adak *et al.*, 1995), but the proportion of human cases caused by contaminated poultry meat is unclear, because there are numerous other potential sources (Siemer *et al.*, 2004), including pets, environmental contamination, contamination of vegetables and bottled water and foreign travel (Evans *et al.*, 2003; Gillespie *et al.*, 2003; Neimann *et al.*, 2003). Community outbreaks are rare, but are often related to contamination of milk (Gillespie *et al.*, 2003). As well as acute disease, *Campylobacter* infection may be associated with long-term sequelae such as Guillain-Barré Syndrome, reactive arthritis and immunoproliferative small-intestinal disease (Lecuit *et al.* 2004).

The large volumes of poultry meat and eggs consumed and the potential for cross-contamination of uncooked products, particularly in catering establishments, makes control of *Salmonella* and *Campylobacter* in poultry a high priority (Lee, 1974; Bryan and Doyle, 1995; Gillespie *et al.*, 2003).

5.3 The prevalence of pathogens on poultry farms

Only data for *Salmonella* and *Campylobacter* will be considered in this section, since these are the two foodborne pathogens for which the determination of prevalence is relevant in relation to control targets. It is difficult to compare prevalence for different situations, since the results obtained are highly dependent on the type of flock and its housing, the sampling and sample-handling methods and the culture or test methods (WHO, 2002). Thus, it is best to consider prevalence on a flock basis, rather than in terms of individual birds, since this can vary considerably for *Salmonella*, according to the time from

infection, housing system and prevailing stress factors. In the case of *Campylobacter*, the individual bird prevalence is high from a few days after the acquisition of infection. What is probably more relevant is the total or mean number of organisms in the flock, which will translate into infectious doses for humans, but there is less information on this aspect.

5.3.1 Prevalence of *Salmonella*

*S.*Enteritidis is predominant in laying flocks and *S.*Typhimurium is relatively uncommon in most countries. There are large variations in the figures in Table 5.1, which partly reflect sampling methods: 300 spent hens per flock is likely to give far greater sensitivity than only 20. In general, the level of *Salmonella* in Sweden, Finland and Norway is very low, because of long-standing, strict control policies, although there has been an increase in prevalence more recently (EC, 2003d). No data are available for Mediterranean countries, but infection rates are thought to be high in some areas and, in particular, eggs imported into the UK from Spain have been highly contaminated with *S.*Enteritidis. Schluter *et al.* (1994) recorded an individual bird prevalence of 7.2% in pullets, 13.3% in laying birds and 6.2% in broiler flocks. Table 5.2 shows data relating to broiler flocks.

There is little reliable prevalence data for turkey, duck and goose flocks (EC, 2003d). The incidence of *Salmonella* in turkeys is thought to be low in Finland, Sweden, Norway, Netherlands and Ireland, but this may not be the case with

Table 5.1 Flock prevalences of *Salmonella* in commercial layers

Country	Prevalence	Number of flocks tested	Sample type	Reference
USA	35%	711	300 spent hens/flock	Hogue *et al*. (1997)
Canada	3%	295	60 faeces/ 20 egg belt swabs	Poppe *et al*. (1991)
Japan	5%	37	20 spent hens	Sunagawa *et al*. (1997)
Denmark	2% [0]	422 [36]	100 spent hens	Gerner-Smidt and Wegener (1999)
Denmark	4.1%	946	NS	EC (2003d)
Finland	0% [0]	1805 [452]	NS	EC (2003d)
Ireland	0.7% [0]	436 [34]	NS	EC (2003d)
Sweden	0.4% [0]	1117 [34]	NS	EC (2003d)
Norway	0% [0]	1708 [32]	NS	EC (2003d)
N. Ireland	25.4%	179	faeces/dust	McDowell (2004)

NS = not specified; [ ] = layer breeders (EC, 2003d).

Table 5.2 Flock prevalences of *Salmonella* in broilers

Country	Prevalence	Number of flocks tested	Sample type	Reference
Canada	76.9%	294	environmental samples	Poppe *et al*. (1992)
USA	5.2%	155	faeces	Jones *et al.* (1991)
Netherlands	24.1%	141	caeca	Goren *et al.* (1988)
Netherlands	31.8%	192	NS	EC (1998)
Austria	3.4%	5029	cloacal swabs	EC (1998)
Denmark	6.5%	4166	'socks'	EC (1998)
	[1.1]	[558]		
France	69.8%	86	faeces	Rose *et al.* (1999)
Germany	4.2%	455	NS	EC (1998)
Ireland	20.7%	1732	NS	EC (1998)
	[5.0]	[187]		
Italy	3.1%	1093	NS	EC (1998)
Norway	<0.01%	2639	NS	ARZN (1998)
	[1.2]	[86]		
Sweden	0.03%	2935	faeces	EC (1998)
	[0]	[291]		
UK	18.5%	3073	litter	Anon (1998)
Finland	0.6%	2954	NS	EC (2003d)
	[0]	[50]		
Austria	1.2%	5453	NS	EC (2003d)
	[0.9]	[795]		

NS = not specified; [ ] = layer breeders (EC, 2003d).

ducks. *S.* Typhimurium, including the DT104 complex, appears to be more common in turkeys than in broilers. In Canada, the high prevalence of 86.7% of 270 flocks found positive in 1990–1991 was associated with contaminated feed (Irwin *et al.*, 1994).

Table 5.3 shows the distribution of the top 10 serovars in chickens in Great Britain. These mostly result from private monitoring of broiler flocks and predominantly reflect serovars that are endemic in feedmills, hatcheries or farms within individual companies. Isolations of *S.* Enteritidis have been rising recently and this mainly reflects an increase in reports from commercial laying flocks.

5.3.2 Prevalence of *Campylobacter* in broiler flocks

Table 5.4 shows some comparative data from different countries. In most countries, flock prevalences are relatively high, but are lower in those countries, such as Finland, Sweden and Norway, that have more rigorous control policies and smaller, less-integrated poultry industries. In Sweden, in recent years, the flock prevalence has been reduced from 50% to 10% and 60 farms are consistently free of *Campylobacter* (EC, 2000).

Table 5.3 Top ten *Salmonella* serovars in British chickens (Jan–Dec 2001–2003)

January–December 2001			January–December 2002			January–December 2003		
Rank	Serovar	Number	Rank	Serovar	Number	Rank	Serovar	Number
1	Senftenberg	144	1	Livingstone	122	1	Livingstone	120
2	Livingstone	76	2	Senftenberg	107	2	6,7:-:-	112
3	Liverpool	59	3	6,7:-:-	67	3	Senftenberg	70
4	Mbandaka	58	4	Kedougou	60	4	Virchow	68
	Thompson	58	5	Montevideo	56	5	Montevideo	52
5	Montevideo	56	6	Binza	54	6	Mbandaka	49
6	Typhimurium	53	7	Mbandaka	51	7	Kedougou	48
7	Heidelberg	50	8	Virchow	47	8	Enteritidis	36
8	6,7:-:-	44	9	Ohio	38	9	Ohio	34
9	Kedougou	42	10	Typhimurium	35	10	4,12:d:-	28

Number = number of incidents (i.e. reported cases, not isolates).

Table 5.4 Prevalences of *Campylobacter* in broilers

Country	Prevalence	Number of flocks tested	Sample type	Reference
Netherlands	30.0%	499	NS	Bouwknegt *et al.* (2004)
Netherlands	82.0%	187	caecal contents	Jacobs-Reitsma *et al.* (1994)
UK	68.0%	93	caecal contents	Humphrey *et al.* (1994)
Sweden	12.0%	3000	cloacal swabs	Berndtson and Engvall (1994)
Norway	18.0%	176	cloacal swabs	Kapperud *et al.* (1993)
Finland	27.0%	49	caecal contents	Aho and Hirn (1988)
Canada	47.0%	60	caecal contents	Prescott and Gellner (1984)
Denmark	42.5%	8911	cloacal swabs	Wedderkopp *et al.* (2001)
France	42.7%	75	NS	Refrégier-Petton *et al.* (2001)
Japan	45.0%	NS	NS	Tanaka *et al.* (1986) cited by Newell and Fearnley (2003)
Denmark (2001)	41.9%	6054	NS	EC (2003d)
Finland (2001)	4.0%	1069	NS	EC (2003d)
Sweden (2001)	16.2%	4220	NS	EC (2003d)
Netherlands (2001)	16.3%	123	NS	EC (2003d)
N. Ireland (1999)	21.6%	194	NS	EC (2003d)

cited by van de Giesen (1996)

NS = not specified

Table 5.5 The chicken breeding and production hierarchy (approximate numbers of potential descendants from one pure-line ancestor)

	Generation			Commercial		
Primary breeding flock	Great-grandparent	Grandparent	Parent	Birds	Tonnes	Eggs
Meat type						
Chickens						
Male line ♂		100	1800	2×10^6	4000	
Female line ♀	9	240	6000	780 000	1560	
Egg layers ♂	—	480	400 000	300×10^6	—	7.2×10^{10}
♀	—	40	3000	240 000	—	5.8×10^7

Adapted from Hunton (1993).

5.3.3 Relevance of *Salmonella* and *Campylobacter* in different poultry sectors

Salmonella is a robust organism that can be transmitted vertically or pseudovertically by survival of organisms on faecally contaminated eggs. The occurrence of *Salmonella* high in the breeding pyramid can therefore lead to widespread infection throughout the production system (Hensel and Neubauer, 2002). Table 5.5 shows the exponential multiplication of birds and products from a single, primary breeding bird. This means that the occurrence of any *Salmonella* at primary breeding or grandparent level is potentially serious and should not be tolerated. In North European countries, such flocks are extensively tested and removed from production, if infection with any *Salmonella* serovar occurs. The situation in relation to turkey and duck flocks is less clear, and, in the UK, there is no statutory requirement for monitoring, but *Salmonella* incidents reported from such breeding flocks are rare.

Only a small number of *Salmonella* serovars are considered to be intrinsically invasive. *S.*Gallinarum/Pullorum is a prime example of this but, being host-adapted, is not a significant foodborne pathogen. *S.*Enteritidis has a special affinity with ovarian and oviducal tissue (Thiagarajan *et al.*, 1994; Keller *et al.*, 1995; de Buck *et al.*, 2004) and is transmitted relatively well via eggs. *S.*Typhimurium, *S.*Virchow, *S.*Infantis and *S.*Hadar may also be classified as potentially invasive and are considered to present the most significant risk to consumers. These particular serovars are subject to special monitoring and control measures within the EU.

Although any *Salmonella* serovar can pass through the hatchery to infect chicks, many are relatively insignificant in terms of human infection, despite being found frequently in broiler flocks. These serovars appear to be adapted to the environments of feedmills, hatcheries and poultry houses so, although they may be difficult to eradicate, they rarely represent a significant risk. In some cases, endemic infections with serovars of low pathogenicity may even be an advantage, if they provoke an immune response or act in a competitive manner against more invasive *Salmonella* serovars, e.g. *S.*4,12:d:- versus *S.*Typhimurium.

In laying flocks, only *S.*Enteritidis is likely to occur regularly in the contents of intact eggs, if the flock is infected, and even *S.*Typhimurium is rarely found (Baker *et al.*, 1980; Okamura *et al.*, 2001). Other serovars may occur as a result of faecal contamination of eggshells, but this is not a frequent source of infection, if eggs are handled correctly in domestic kitchens and catering establishments. Despite the theoretical difficulties in maintaining biosecurity for free-range flocks, industry data suggest that both free-range broiler flocks and laying flocks are less likely to be infected with *Salmonella* than conventionally-housed flocks (McDowell, 2004). The significance of *Salmonella* in turkey production is more difficult to assess (Hird *et al.*, 1993) and, in some respects, there is a closer similarity between turkey and human serovars than there is for chickens and humans (VLA, 2003). More comparative molecular studies are needed to clarify this issue. Although *S.*Enteritidis and *S.*Typhimurium are

frequently reported from ducks, the phage types and antimicrobial-resistance profiles found are uncommon in humans and other, predominantly 'duck' serovars are also rare in human cases.

Campylobacter, especially *C. jejuni*, is commonly carried by a wide range of domestic livestock, wildlife and pets. Although it occurs in high-biosecurity, primary breeding flocks, this is unlikely to be significant, since vertical and hatchery-derived transmission are not thought to be common transmission routes. *Campylobacter* can be excluded from housed poultry for much of the time, when strict biosecurity is properly applied, but such measures commonly break down in hot summer weather (Wedderkopp *et al.*, 2000). Biosecurity is impossible for free-range flocks. *Campylobacter* also occurs in laying flocks, although it may be less prevalent in caged birds. Since there is no ovarian transmission of *Campylobacter*, and survival of the organism on eggshells is poor, infection of laying flocks is not relevant to human foodborne disease.

5.4 Sources of infection

5.4.1 *Salmonella*

Feed

The extent of *Salmonella* contamination of feed is substantially underestimated, because of the difficulties in obtaining representative samples, when monitoring ingredients or finished feed (Durand *et al.*, 1990; Schluter *et al.*, 1994). The presence of *Salmonella* in feed can lead to wide distribution of the organism within a poultry company and beyond (Chadfield *et al.*, 2001). Most contamination of feed currently originates from vegetable-protein ingredients, which have been contaminated during growing, harvesting or storage in the country of origin. Specific *Salmonella* serovars in highly contaminated consignments of oilseed may contaminate the cooling systems used for oilseed meal. Contaminated product is then supplied to feedmills and may lead to production of contaminated meals or further contamination of coolers used for pelleted or heat-treated meals. *Salmonella* Enteritidis and *S.* Typhimurium are rarely detected in animal feeds (VLA, 2003), but may be found in dust from grain-handling and storage areas of mills, indicating that some otherwise undetected contamination may be occurring (Davies and Wray, 1997). Wildlife-related serovars are also found commonly in these grain-handling areas. Contaminated dust presents a recontamination risk for heat-treated feed (Jones and Richardson, 2004). Although feed has been dismissed as a major source of *Salmonella* in recent years, because of the low prevalence in finished feeds and ingredients, it is still the principal means of introducing new serovars into integrated poultry companies (Bailey *et al.*, 1999), and the organisms then become established in the company mill, hatchery or problem farms (Wilson, 2002). Many *Salmonella* serovars detected in feed are not found frequently in humans, but some outbreaks have been traced to an original incident of feed contamination (Moore *et al.*, 2003). Because of its sensitivity to dry conditions,

Campylobacter is unlikely to survive for long in feed (Whyte *et al.*, 2003), although fresh pigeon faeces may be deposited onto feed by vibration of the bulk loading gantry in some mills (Davies and Wray, 1997). A more detailed consideration of feed contamination is presented in Chapter 7.

Hatchery

The hatchery is a focal point for any contamination that may be present on the surface of, or within, eggs (Bhatia and McNabb, 1980; Cox *et al.*, 1990; Davies and Wray, 1994; Davies *et al.*, 1997, 2001). Once introduced into the hatchery, the incubation conditions can amplify and disseminate the organisms, so that a low prevalence of contaminated eggs from one breeding flock may lead to infected chicks that are distributed to a number of commercial farms (Skov *et al.*, 1999a; Liebana *et al.*, 2002). This cross-contamination occurs primarily in hatcher incubators but, in some cases, there may also be contamination of the setters, particularly if these are multistage machines, egg-transfer and chick-handling equipment, and automated tray-washers. Waste-handling areas, where debris is collected from hatcher baskets, and maceration and washing areas can also be a source of recontamination via aerosols and surface water.

Table 5.6 shows the distribution of *Salmonella* in five chicken and one duck hatchery investigated by the author by taking surface-swabs from equipment. This indicates significant contamination, despite little evidence of *Salmonella* in routine monitoring of dead-in-shell chicks. It is essential to monitor the process regularly (Bailey *et al.*, 2001). Control of *Salmonella* in hatcheries has become more difficult in recent years, since the health and safety policies operated by poultry companies have led to the use of less noxious, but also less effective, disinfectants (Mitchell and Waltman, 2003). These include amphoteric surfactants, peroxygens and quaternary ammonium compounds, which may be more easily inactivated by residual organic matter. Failure of cleaning and disinfection regimes allows any *Salmonella* present to become established, particularly in ventilation-ducting and beneath the door-seals of hatcher incubators. The disinfectants may also be ineffective at standard concentrations in tray-wash machines, so there is a risk of contaminating hatcher baskets, delivery baskets and egg trays, if the concentration is not properly controlled at effective levels (Davies *et al.*, 2001). In some other hatcheries, poor design or expansion of the premises has led to inappropriate flows of eggs, chicks and air, which allows recontamination of cleaned areas. The endemic contamination of hatcheries may involve multiple serovars simultaneously, but often there are one or two predominant serovars that can persist for years (Byrd *et al.*, 1999). The situation is quite complex, as some serovars found in breeding flocks may never be detected in the hatchery (Bailey *et al.*, 2002), others found in the hatchery may have no obvious link with breeding flocks, while some serovars may be transient residents of the hatchery and others more permanent. Not all serovars found in the hatchery are significant colonisers of the resultant chicks. It is also clear that diversification of *Salmonella* serovars in terms of phage type and genotype can occur during their presence in a hatchery (Davies *et al.*, 2003).

Table 5.6 Distribution of *Salmonella* contamination in six poultry hatcheries (Davies and Breslin, 2004b)

Hatchery	Egg handling and storage	Setter incubator areas	Egg transfer area	Hatcher areas	Chick handling area	Tray wash areas	Waste areas	Main ventilation ducting	Miscellaneous stores, vehicles, etc.
1 (C)	5/29 (17.2) a,b,c^{2},d	1/61 (1.6) c	2/11 (18.2) a,d	30/70 (42.9) c,d^{26},e^{2},f	5/27 (18.5) c^{4},d	1/23 (4.3) d	3/6 (50.0) c	0/10	7/28 (25.0) c^{4},e,g,h
2 (C)	0/23	0/74	6/15 (40.0) i^{2},j^{4}	31/99 (31.3) i^{12},j^{18},k	13/19 (68.4) g^{2},i^{4},j^{2},k	0/12	1/1 g	2/6 (33.3) g^{2},j	6/15 (40.0) j
3 (C)	1/65 (1.5) g	0/40	0/26	4/56 (7.1) c^{3},l	9/58 (15.8) c	6/32 (18.7) c^{5},m	8/9 (88.9) g,c^{6},l	0/10	0/44
4 (D)	12/43 (27.9) n^{4},o^{3},q^{3},r^{3}	15/56 (26.8) b,n,o^{5},p^{4},q,r^{3}	4/15 (26.7) n,r^{3}	20/27 (74.1) q^{18},r^{2}	81/119 (68.1) b^{6},c^{13},m^{13},o^{7},q^{41},r	6/30 (20.0) n^{3},o,r^{2}	3/6 (50.0) n^{2},o	5/6 (83.3) o^{2},q^{3}	4/9 (44.4) q^{3},r
5 (C)	1/22 (4.5) f	10/34 (29.4) f	1/15 (6.7) f	26/108 (24.1) f	4/74 (5.4) d,f^{3}	5/19 (26.3) d,f^{4}	5/10 (50.0) f	5/8 (62.5) f	1/24 (4.2) f
6 (C)	0/29	0/35	0/5	17/125 (13.6) g^{7},s^{10}	5/36 (13.9) g	8/36 (22.2) g^{5},s^{3}	9/10 (90.0) g	0/4	1/23 (4.3) s

Data presented as number of samples positive for *Salmonella*/number of samples taken (%)
Key: C chicken; D duck
Salmonella serotype key: a New Brunswick; b Enteritidis PT untypable; c Enteritidis PT6; d Senftenberg; e Binza; f Virchow; g Enteritidis PT4; h Enteritidis PT21; i Livingstone; j Thomasville; k Mbandaka; l Enteritidis PT34; m Enteritidis PT3; n Enteritidis PT96; o Typhimurium DT8; p Typhimurium DT30; q Enteritidis (not phage typed); r Typhimurium (not phage typed); s Montevideo.
Number after *Salmonella* type letter is the number of isolates of each serotype/phage type.

5.4.2 *Campylobacter*

Manufactured feed is considered too dry to allow survival of any campylobacters and hatchery infection is not thought to be a significant source of the organisms. Evidence for the entry of *Campylobacter* into hatcheries has been obtained by the polymerase chain reaction (PCR) (Hiett *et al.*, 2002), but not by culture. In some studies, similar genotypes of *C. jejuni* were found in breeding and associated broiler flocks (Cox *et al.*, 1999, 2002a), but not in other work (Petersen *et al.*, 2001), where numerous clones occurred in breeding flocks, so apparent associations may reflect sampling variation or a common environmental/fomite source. Although *Campylobacter* may be found on fresh eggs, its survival is poor, unlike *Salmonella* (Sahin *et al.*, 2003a). The lower prevalence of *Campylobacter* in winter and the occurrence of mainly *Campylobacter*-free farms within an integrated company would also be incompatible with the hatchery being a significant source (Shane, 2000).

5.4.3 Vertical transmission

True vertical transmission involves the deposition of organisms into forming eggs, either in the ovary or during formation of the shell, as the egg passes down the oviduct. For *Salmonella*, this occurs more usually with invasive serovars, such as *S.*Gallinarum/Pullorum (Rabsch *et al.*, 2000), which commonly cause systemic infections, and *S.* Enteritidis, which can become localised and persist, sometimes for the whole life of the bird, in glandular parts of the reproductive tract, including the magnum, isthmus and ovarian granulosa cells (O'Brien, 1990; Thiagarajan *et al.*, 1994; Keller *et al.*, 1995; Guard-Petter, 2001). Other invasive serovars, such as *S.* Typhimurium, may be deposited in eggs during the septicaemic phase of infection, but do not have a special ovarian association and so produce only a low frequency of internally contaminated eggs for a short period after infection (Baker *et al.*, 1980; Okamura *et al.*, 2001). Chicks from internally contaminated eggs are likely to hatch already infected, while shell fragments and fluff released during hatching will also be more contaminated than those derived from external egg contamination.

Pseudovertical transmission may occur with any *Salmonella* serovar. This involves internalisation of *Salmonella* from the faecally-contaminated surface of the egg during the cooling phase immediately after lay, and before the cuticle has formed. Cracked eggs may also admit *Salmonella* and be a cause of chick infection and incubator contamination. Highly contaminated eggs that have been poorly sanitised may occasionally allow survival of viable organisms on the shell surface during incubation.

Although *Campylobacter* may colonise the oviduct of laying hens, this is considered to be due to ascending faecal contamination (Camarda *et al.*, 2000; Modugno *et al.*, 2000) and true vertical transmission has not been demonstrated experimentally. The organism has also been found in the genital tract of male breeding chickens (Hiett *et al.*, 2003) and in semen (Cox *et al.*, 2002b), but may

involve cross-contamination. Penetration of eggs and survival within the egg is generally poor (Sahin *et al.*, 2003a).

5.4.4 Persistent farm contamination

A major source of *Salmonella* infection in poultry is contamination of the farm environment, particularly that remaining inside houses and on equipment after cleaning and disinfection (Rose *et al.*, 1999, 2000). This situation is common with less invasive serovars, particularly those originating from feed (Limawongpranee *et al.*, 1999), which may become environmentally adapted. Invasive serovars, especially *S.* Typhimurium, are less likely to persist on farms or in hatcheries and feedmills (Chriél *et al.*, 1999), and infected breeding flocks or other animal sources are more important. *Salmonella* originating from the flock may also persist outside poultry houses in anterooms or in dust and litter from the house. Wildlife with access to such materials may then become infected (Davies *et al.*, 2001). In the USA, routine cleaning and disinfection of broiler houses is not carried out after every crop but, despite this, it is not inevitable that new birds placed in a contaminated house will become infected. Poor cleaning and, in particular, insufficient disinfection may increase the risk of infection by distributing the organisms and leaving a damp environment.

The presence of breeding colonies of mice within poultry houses is a major factor that contributes to the chances of infection (Henzler and Opitz, 1992; Garber *et al.*, 2003; Liebana *et al.*, 2003). This is not a common problem in modern broiler or breeder/layer rearing houses, as there is little harbourage of, or easy access to, feed. In broiler-breeder farms, on the other hand, there may be considerable harbourage in mini-pits and easy access to feed in slave hoppers. There is a similar risk in barn or free-range egg production, but it is in cage houses that mice are particularly persistent, because they can breed in droppings pits or house insulation and readily gain access to feeders, without interference from the birds.

Persistence of *S.* Enteritidis and, to a lesser extent, other serovars on laying farms is a considerable problem, because of multi-age sites. Unlike breeding, rearing and broiler farms, these farms are occupied continuously, so there is always a source of feed, contaminated dust and other materials, as well as operatives that may rapidly infect newly-housed flocks. The risk is greatest when houses are linked by common egg belts and dung belts/channels (Davies and Breslin, 2003a). Typically, normal cleaning and disinfection of caged-layer houses and pest control are insufficient to eliminate *Salmonella*, and much reliance is placed on vaccination against *S.* Enteritidis to suppress infection (Berghold *et al.*, 2003; Davies and Breslin, 2003b). In Denmark, prolonged heating of cage houses, using steam containing formaldehyde, has been used successfully to eliminate infection on problem farms (Gradel *et al.*, 2004).

Persistence of contamination inside conventional broiler houses is not thought to be a major problem with *Campylobacter*, but farms with non-waterproof electrical fittings or roof-mounted extractor fans that are not easily cleaned can be at increased risk, suggesting that there may be some persistence

(Shane, 2000). Organic flocks may become infected at a young age during the housed brooding period, because powerful disinfectants are rarely used (Dr Vivian Allen, personal communication). Occasionally, the organism may be found in poorly cleaned broiler houses (Stern *et al.*, 2001; Davies R.H., unpublished), but it is not always associated with an infection. *Campylobacter* can persist in biofilms in water distribution lines, but is not found in houses holding negative flocks and, in infected flocks, the genotypes in the house are often different from those in the birds (Zimmer *et al.*, 2003). Because *Campylobacter* survives poorly in dry environments and is sensitive to oxygen and disinfectants, it is likely that significant survival occurs only in poorly cleaned and dried houses, where contaminated wash-water is allowed to collect on surfaces or in equipment (Shane, 2000).

5.4.5 Other sources of infection

Salmonella is found in a proportion of free-living wildlife species, but these are not thought to be a major source of infection for poultry flocks, even for free-range flocks, where the incidence of *Salmonella* is lower than in housed flocks. It is possible for the organisms to be carried between farms by wild birds, rats, flies and predatory animals, such as cats, badgers and foxes, but this appears to be more of a risk on cattle and pig farms. Persistence of *Salmonella* outside the poultry house is common after cleaning and disinfection, but does not appear to be strongly related to the risk of infection for flocks housed subsequently (Davies *et al.*, 2001).

Campylobacter, in contrast, is regularly found in large numbers in the faeces of free-living wild animals, especially corvids and gulls (Maruyama *et al.*, 1990; Broman *et al.*, 2002). Such carrier animals may be a source of contamination for the external environment of poultry houses and for free-range farms (Shane, 2000). Arthropods and molluscs may enter poultry houses beneath doors and through air-inlet vents, and may also act as transport vectors, but their relative significance is unknown. It has been reported that, in most cases, the genotypes of *Campylobacter* found in the external environment of the houses and in the flocks are different (Nesbit *et al.*, 2001), but this may be a feature of the sampling regime in the face of a large diversity of environmental strains. It is also possible that some strains that are not predominant in the environment may be overlooked, because of low numbers or competition from other *Campylobacter* strains or competitor organisms. Nevertheless, such strains may be able to colonise birds and then multiply to high numbers, which make them more easily detectable. In some countries, there appears to be a high frequency of *Campylobacter* clones that persist from one flock to another (Petersen and Wedderkopp, 2001), but is not apparent elsewhere (Newell and Wagenaar, 2000). Other infected animals may contribute to contamination of the environment, including water supplies. Cattle are a significant source of *C. jejuni* (Ziprin *et al.*, 2003a) and turn-out of cattle, irrigation of land with slurry, in which *Campylobacter* may survive in large numbers (Horan *et al.*, 2004), and spread of wash-water from dairy farms may lead to higher levels of contamination in the environment

and local water sources at certain times of the year (Eyles *et al.*, 2003; Ross and Donnison, 2003; Stanley and Jones, 2003). Similarly, the spring/summer seasonal peak of *Campylobacter* infection in humans may precede that of poultry (Newell and Fearnley, 2003), so human waste could also be responsible for an increased environmental burden.

Crates and modules used for transporting broiler flocks to slaughter and, in some cases, those taking pullet flocks to laying sites, may be a source of *Salmonella* (Rigby *et al.*, 1982; Heyndrickx *et al.*, 2002) or *Campylobacter* (Berrang *et al.*, 2003) but, because most crates are contaminated after washing and, in some companies, *Salmonella* is rare in broiler flocks, their contribution to *Salmonella* infection is uncertain. In the case of *Campylobacter*, there is a substantial increase in flock infection after thinning (Hald *et al.*, 2000, 2001; Berrang *et al.*, 2003). It is uncertain, however, whether this relates to contamination of the transport crates and modules at the abattoir; contamination of the catching team and their vehicles; introduction of infection from outside the poultry house by the considerable activity involved in thinning; or merely by relaxation of farm hygiene after thinning.

Airborne spread of *Salmonella*, primarily on contaminated dust particles, but also on aerosols produced by waste-handling equipment or washing of adjacent houses on multistage sites, has been cited as a major risk factor (Baskerville *et al.*, 1992). In practice, however, this may only occur in closed air spaces, since individual broiler flocks that are housed next to highly infected flocks often remain free of infection, and, in turkey breeding flocks, infection may be confined to one pen within a house, as long as a separate feeder is used in each pen (Davies, R.H., unpublished). *Campylobacter* can be found in dust at some distance from an infected poultry house, even though its survival in dust is poor. Spread of *Campylobacter* to all houses on a site, when one house becomes infected, is common but does not always occur. More work is needed to determine the relative importance of different environmental sources and means of spread of *Salmonella* and *Campylobacter* in poultry flocks.

Staff handling poultry may become contaminated externally with *Campylobacter* or *Salmonella*, especially if they also deal with other animal species on the farm or elsewhere. There is a low level of carriage of these organisms in healthy humans or those who have recovered from infection. It is not known whether this represents a serious threat, but data from monitoring in the poultry-breeding sector suggests that, on occasion, when sensitive test methods are used, specific *Salmonella* serovars may be found in personnel before they are identified in breeding flocks. Provision of dedicated protective clothing in each house appears to reduce the risk of introducing *Campylobacter* (Bouwknegt *et al.*, 2004).

5.4.6 Behaviour of the organisms in poultry flocks and the environment

Chicks are high susceptible to *Salmonella* in the first week of life and may become infected rapidly, if exposed at the hatchery or when housed. In broiler

and rearing flocks, the individual-bird prevalence usually peaks by two weeks of age and then begins to decline (Dougherty, 1976; Gradel *et al.*, 2002; Heyndrickx *et al.*, 2002). In the case of *S.* Enteritidis, such early infection may gradually become undetectable, until the onset of lay, when latent infections can be re-activated (Davies *et al.*, 1997; Holt *et al.*, 1999). In laying flocks, there is often a fluctuation in the shedding of *Salmonella* over the life of the flock, with increases during periods of stress and towards the end of lay (Davies and Breslin, 2001). In barn and free-range laying flocks, the early peak of infection is usually more transient and, in vaccinated flocks, *S.* Enteritidis may not be detectable later on, although there is usually an increase in shedding at the end of a flock's working life (Davies and Breslin, 2003b).

Campylobacter behaves in a very different manner. The infectious dose is thought to be very low, infection typically occurs from 2–3 weeks of age onwards (Cawthraw *et al.*, 1996) and large numbers of organisms are soon excreted by infected birds (Newell and Fearnley, 2003). This leads to rapid spread of infection, so that virtually 100% of birds in a broiler house may be colonised within a few days (Berndtson *et al.*, 1996a,b). Surprisingly, some birds may remain free from infection, despite the high level of challenge form their cohorts. In the first 2–3 weeks of life, the so-called 'lag phase', infection is unusual and it is also more difficult to infect birds artificially (Shane, 2000; Sahin *et al.*, 2003b; Ziprin *et al.*, 2003b). This lag phase is thought to be largely due to the presence of maternal antibodies (since all parent flocks are infected with *C. jejuni*). The antibodies wane within 3–4 weeks (Sahin *et al.*, 2001). In some flocks, notably organic flocks, infection may occur earlier, and this is thought to be associated with an overwhelming challenge (Dr Vivian Allen, personal communication). Other factors, such as the effects of changes in diet and medication on the developing intestinal flora, may also play some part in flock susceptibility.

Management of the flock (e.g. stress, feed withdrawal) may have an effect on the prevalence of infection and on the numbers of organisms being shed (Corrier *et al.*, 1999; Northcutt *et al.*, 2003). In wild birds, *Campylobacter* infection is self-limiting within a few weeks (Glünder *et al.*, 1992), but, although some reduction in infection may occur towards the end of the life of a broiler or turkey flock, most birds will still be excreting large numbers of organisms (Newell and Fearnley, 2003).

Most serovars of *Salmonella* can be very persistent in the environment and may survive in dust for years (Davies and Wray, 1996a). In litter and pooled faeces, the competitive action of other bacteria rapidly reduces *Salmonella* numbers, but the organism does survive well in thin layers of litter in scratching areas of laying houses or in small portions of litter that become trapped in the walls or floors of poultry houses, or are spilled outside the house (Davies and Wray, 1996a; Davies and Breslin, 2001). *Salmonella* can also survive for months in soil on free-range farms, but this does not appear to result in repeated infections of vaccinated flocks (Davies and Breslin, 2003b,c).

Campylobacter survives poorly in dry materials, since it is sensitive to desiccation and oxygen, and rapidly disappears from litter (Shane, 2000). It

survives longer in water, so pools of water are a good environmental source on poultry farms. Survival in other environmental niches may be underestimated, because of the lack of sensitivity of culture methods (Shane, 2000), although the ability of poorly culturable organisms to cause infection is questionable (Cappelier *et al.*, 1999). Given that the behaviour of *Salmonella* and *Campylobacter* is so different, it is not surprising that there is little correlation between the occurrence of each organism in broiler flocks (Wedderkopp *et al.*, 2001).

5.5 Sampling techniques

Effective sampling and handling of the sample are the most important aspects of *Salmonella* monitoring. Sampling must be planned carefully according to the objective of the test and the likely variation in infected birds (Andersen-Sprecher *et al.*, 1994); in practice, infection may be clustered, especially in laying houses (Riemann *et al.*, 1998; Hayes *et al.*, 2000; Mallinson *et al.*, 2000). Although faeces or tissues taken from individual birds at post-mortem may be preferable for comparative purposes (Altekruse *et al.*, 2003), these are normally insufficiently sensitive and too costly for routine use (Davison *et al.*, 1995), and it is agreed that environmental monitoring, especially at key points of contamination, is superior (Kradel and Miller, 1991). In most types of poultry house, dust is the best sample to take, since competitor organisms do not survive as well as *Salmonella* (Davies *et al.*, 1998, 2003; Davies and Breslin, 2001). In most types of house, accumulation of dust is commonly greatest on extraction vents but, where these are very high or sunk in a pit, dust on ledges close to the birds may be preferable. In houses with automated egg collection, the most productive dust is often the accumulated material from egg elevators or brushes/spillage trays, after belts have been running. In cage houses, dust underneath the cages is an excellent sample and, for houses where pens of breeding birds are divided by solid walk-ways, sweepings from these will often identify adjacent infected pens (Davies *et al.* 1998). Where air filters are used, dust can also be gathered from these, particularly in hatcheries and feedmills (Kwon *et al.*, 2000).

In cage houses, where droppings belts or scrapers are present, the best site to detect faecal contamination is the scrapers, after belts have been run or droppings boards scraped. Where birds are on litter, representative faecal sampling is appropriate, but more laborious. At the Veterinary Laboratories Agency (VLA), gauze swabs (Kleenex Readiwipes) are used (Davies and Wray, 1996b). These are autoclaved in 225 ml amounts of buffered peptone water (BPW) in plastic jars and allow sampling of a large area of the litter surface or of pooled faeces on ramps, pop-holes and weighers. Some workers claim that cellulose sponge swabs, pre-soaked in maximum recovery diluent, are superior (Österblad *et al.*, 2003). If the samples are not to be cultured on the same day, then maximum recovery diluent should be used and the swabs kept cool until cultured. Many poultry companies take samples of pooled litter or faeces, but it

is essential that these are collected in a representative manner and mixed well before sub-sampling (Cannon and Nicholls, 2002). One approach is to create a slurry, using an equal volume of BPW, mix well then add 50 ml of the slurry to 200 ml of BPW for subsequent culture. It is best to mix pooled samples manually, with care, rather than blend or 'stomach' them, as this process can release inhibitory factors and directly damage low numbers of organisms. The same considerations apply to egg contents (Seo *et al.*, 2003). For best detection of *Salmonella* in faeces, it may be beneficial to culture an additional 1:10 dilution of the initial sample, since, at higher concentrations, particulates and other competitive factors may influence the test result. With other samples, such as dust, feed or carcasses (Simmons *et al.*, 2003), or where direct selective enrichment of faeces is used, the larger the volume of material, the more sensitive the test (Funk *et al.*, 2000).

Indirect methods of sampling faeces have been devised. These usually take the form of drag swabs or boot swabs/socks. Originally, drag swabs consisted of three large, moistened gauze pads, pulled behind a stick, along the length of a poultry house or droppings pit (Mallinson *et al.*, 1989). Such swabs are an effective means of sampling (Kingston, 1981), but the sampling assembly is cumbersome in occupied houses, and good technique and subsequent handling of samples is crucial (Opengart *et al.*, 1991; Opara *et al.*, 1992). Commercial attempts to scale down the drag swab do not produce equivalent results, since smaller swabs become saturated more quickly (Rolfe *et al.*, 2000). A single drag swabbing is not sufficient to identify an infected flock in all cases, and the identification of positive flocks increases in proportion to the number of assemblies used (Caldwell *et al.*, 1994). It has been claimed that drag swabs are more sensitive than boot swabs (Caldwell *et al.*, 1998), but this depends on the situation being sampled, the type and method of drag swabbing and the type and method of boot swabbing. Some boot swabs are insufficiently absorptive, but others work well (Aho, 1992; Heyndrickx *et al.*, 2002). In Denmark, a tube of gauze bandage has been used as a boot or 'sock' swab and 2–5 pairs per house have given reasonable sensitivity, compared with testing large numbers of individual faeces in pools (Skov *et al.*, 1999b; Gradel *et al.*, 2002). For monitoring non-caged flocks, a combination of boot swabs and dust is the most practical and sensitive method, and initial sampling is best carried out about three weeks after housing to correspond with the accumulation of salmonellas from the early peak of infection (Gradel *et al.*, 2002; Heyndrickx *et al.*, 2002). At the end of the crop, wash-water from the house provides a sensitive pooled sample (Fu *et al.*, 2001) and it is essential to sample effectively after cleaning and disinfection of a contaminated house (Davies and Wray, 1996b).

Sampling in the hatchery is important, and culture of only dead-in-shell or cull chicks will significantly underestimate contamination problems (Hafez and Jodas, 1992). Many poultry companies also culture hatcher fluff samples, but hatcher and delivery-box liners and macerated waste are better sources of contamination. If positive, it will be necessary to increase flock-specific or incubator-specific sampling to identify the source of the infection. Sampling in

feedmills and abattoirs also needs to be upgraded, as these can act as sensitive sentinels for the appearance of new *Salmonella* serovars within a population. Thus, dust accumulating in bins and auger systems, around equipment and in coolers in different parts of feedmills can reflect contamination of the ingredient or finished-product area, while unloading and waste areas in poultry processing plants can yield *Salmonella* serovars originating from processed flocks.

Sampling for *Campylobacter* has been less well researched than that for *Salmonella* (Reilly and Gilliland, 2003), since the high individual-bird prevalence and large numbers of organisms excreted by infected birds means that detection is usually straightforward (Newell and Fearnley, 2003). However, low-prevalence infection can occur after thinning, before there has been a chance for widespread infection to occur. Cloacal swabs or caecal contents are normally used for monitoring by poultry companies, but fresh droppings, particularly caecal droppings, may give better results and boot swabs are increasingly used in research studies (Stern and Robach, 1995; Eifert *et al.*, 2003; Dr Vivian Allen, personal communication). It is essential that samples are maintained in a moist condition away from air, so cloacal swabs in charcoal transport medium, collected directly into culture media, or full, tightly-closed faeces sample pots, with no air space, are required. *Campylobacter* in water supplies is best detected by culture or PCR tests carried out on filters used to process large volumes of water.

5.6 Control of *Salmonella*

5.6.1 Statutory controls

The EU Zoonoses Directive currently specifies monitoring and control measures that are then enacted by specific legislation in Member States. Table 5.7 outlines the legislation in place in the UK, which currently applies mainly to breeding chickens (Stewart, 1999), but which will be extended in future to commercial laying flocks, broilers and turkeys. Statutory controls are normally supplemented by government and industry codes of practice (Anon., 2002).

Some of the Scandinavian countries, such as Sweden, Norway and Finland, have a zero-tolerance policy for *Salmonella* in poultry production (Wierup *et al.*, 1995) and infected flocks and their products will be heat-treated or destroyed, if any *Salmonella* serovar is found. This pathogen-free approach is not possible in most countries, but, in Denmark, monitoring and control have become much stricter in all poultry sectors (Bisgaard, 1992; Wegener *et al.*, 2003) and some poultry meat is sold as 'Salmonella-free'. The basis of *Salmonella* control is biosecurity – to stop *Salmonella* entering the farm and to eliminate it quickly if it should enter the holding (Humphrey, 1989). This approach should apply throughout the production chain to avoid dissemination of the organisms (McIlroy *et al.*, 1989; Anon., 1991; Forsythe, 1996; Noordhuizen and Welpelo, 1996; Johnston, 2000).

Biosecurity is defined as a health plan for measures designed to protect an animal population from transmissible infectious agents (Anon.,1999). This

Table 5.7 *Salmonella* control legislation

Mandates	Description
Domestic legislation: specific to *Salmonella*	The Zoonoses Order of 1989 makes the laboratory detection of *Salmonella* infection in animals used for food production reportable. It also gives power to restrict the movement of any *Salmonella*-infected animals that are considered to constitute a high risk to human health. The Order gives powers to take samples and also provides for compulsory slaughter, with compensation and subsequent cleansing and disinfection of the premises.
	The Poultry (Seizure of Hatching Eggs) Order of 1990 gives powers to seize and dispose of any hatching eggs in order to prevent the spread of *Salmonella*. The Poultry Breeding Flocks and Hatcheries Order of 1993 requires any but the very smallest chicken breeding flock or hatchery to register and provide routine samples for *Salmonella* testing at an approved laboratory. Breeders of poultry other than domestic fowl can join the monitoring scheme voluntarily.
	The Animal By-Products Order of 1999 requires animal by-products to be disposed of by rendering at approved premises, or by incineration or burial. There is also a requirement to stain any products in transit to avoid them being diverted in error into any animal or human food chain.
	The Importation of Processed Animal Protein Order of 1981 permits the importation of protein of animal origin under licence. It gives powers for imports to be sampled for *Salmonella*. Importation can be banned from countries where products are persistently contaminated with *Salmonella*.
OIE requirements	*Salmonella* Abortusovis – List B (Sheep & Goat Diseases) *Salmonella* Gallinarum (Fowl typhoid) – List B (Avian Diseases) *Salmonella* Pullorum (Pullorum Disease) – List B (Avian Diseases)
EU requirements	Directive 2003/99/EC requires monitoring of poultry breeding flocks and hatcheries for *S*. Enteritidis and *S.* Typhimurium, as well as other serotypes of public health significance. Member States are to submit a control programme for approval and to monitor prevalences and set targets for future improvements in *Salmonella* in poultry and pigs.
Other guidance documents	Codes of Practice have been published by DEFRA that give advice on prevention and control of *Salmonella* in several farmed species. Representative bodies of the egg industry and pig industry have also included monitoring for *Salmonella* as a condition of membership of their assurance schemes. Monitoring of broiler and major turkey flocks for *Salmonella* is usual, as a result of the requirements of assurance schemes of major retailers.

embodies all measures which can or should be taken to prevent viruses, bacteria, fungi, protozoa, parasites, disease carriers (rodents, insects, wild birds, people, equipment, etc.) from entering and endangering the health status of the population.

In the poultry industry, biosecurity measures are used to minimise the risk of *Salmonella* entering poultry farms and associated enterprises, such as feedmills and hatcheries. Comprehensive biosecurity measures are costly in terms of capital equipment, use of disinfectants and other antibacterials, testing and labour. The maximum level of biosecurity is only possible where there is a high-value product and the consequences of *Salmonella* being transmitted to customers are severe. Such measures are normally applied in full only for primary breeding and grandparent flocks, and include heat treatment of feed at higher temperatures and for longer periods than those used in the commercial sector, combined with the addition of effective organic acid products at suitable concentrations. Feed is often tested for *Salmonella*, using rapid methods, before delivery to farms, and at feedmills there is monitoring of the process and production environment, as well as testing of ingredients and finished products. There is extremely frequent and comprehensive monitoring for *Salmonella* on farms and in hatcheries, and staff are also monitored. They are not allowed contact with the birds whilst excreting *Salmonella*. Visitors may also be asked to provide a negative faecal test result before being allowed on the premises. Entry to the premises is via a hygiene barrier, where showering in and out and use of disposable or site-dedicated protective clothing is required. Equipment used by contractors is a high risk (Heyndrickx *et al.*, 2002; Pattison, 2003) and is either supplied by the company or fumigated on entry to the farm. Other farm inputs, such as litter, are carefully sourced to minimise risk, tested and usually treated with antibacterial substances, such as organic acids or formaldehyde/acid combinations.

One of the basic principles of effective biosecurity in the commercial sector – all-in/all-out production on a whole-farm basis – is often not possible on primary breeding farms, because of the need to maintain and evaluate small groups of birds of high genetic potential. This presents a severe risk from possible persistence of *Salmonella* and hence the high level of biosecurity. This strict biosecurity applies in broiler, primary and grandparent breeding in most North European countries, but measures may be less strict in grandparent flocks of some layer breeders, turkeys and ducks (Davies *et al.*, 1998, 2003), where there may be farms or hatcheries that are not completely dedicated to grandparent production.

At parent level, in conventional, but not organic production, all-in/all-out production is normal. Many of the biosecurity principles described above are applied, but at a lower intensity, because of cost. Although chicks originating from grandparent flocks are free from *Salmonella*, they may become infected during the rearing or laying stages through any of the routes described above, particularly via contaminated feed, except possibly in the case of *S.* Typhimurium and *S.* Enteritidis, which are rarely found in feed, but can be

found regularly in the grain-storage areas of feedmills (Davies and Wray, 1997). Currently, the predominant cause of parent-flock infection with *S.* Enteritidis and *S.* Typhimurium is unknown, but there are significant levels of both organisms in the human and animal populations, and high levels of *S.* Enteritidis can still be found on some commercial laying farms in most countries, and in broiler production in some EU Member States.

All-in/all-out production should ensure that *Salmonella* does not persist for more than one flock cycle, since it is possible to depopulate farms completely, remove all contaminated material, wash, disinfect and test, to ensure that decontamination has been successful, before restocking the houses. In practice, there is sometimes insufficient time to complete the whole process effectively, before restocking. In particular, carriage of *S.* Enteritidis and, to a lesser extent, *S.* Typhimurium and other serovars in breeding mouse populations, has resulted in a high level of persistent infection. The mice are harboured in minipits, storage areas and wall and roof insulation within the house. In the UK, the introduction of vaccination in the broiler-breeder sector, combined with improved hygiene and biosecurity, was fundamental in breaking the cycle of persistent farm infection, hatchery contamination and dissemination of *S.* Enteritidis. In the parent layer-breeder sector, vaccination is not used regularly, since widespread and persistent infection of flocks have not been such a problem.

For commercial broiler production in the UK, improvements in the *Salmonella* status of breeding flocks and feed, and improved cleaning and disinfection of houses has reduced *Salmonella* to a low level. At this time, there is considerable interest in further improving on-farm biosecurity to reduce the prevalence of *Campylobacter* and possible introduction of viral diseases, such as avian influenza. These measures include dedicated boots (and, in some cases, protective oversuits) for each house, improved facilities and protocols for hand hygiene, step-over barriers between a ‘clean’ and ‘dirty’ part of the service area or ante-room and improved tidiness outside the house. Such improvements also include in-filling of areas where water can pool and better drainage. Biosecurity in large-scale turkey production is of a similar standard, but there are considerable problems in applying biosecurity on commercial duck farms and laying farms (especially multi-age, caged laying flocks). On caged layer farms, movement of mice and other rodents, flies, egg belts and personnel can spread *S.* Enteritidis between houses, despite vaccination (Davies and Breslin, 2003b). Mice and poor cleaning and disinfection are also responsible for persistence of salmonellas on the farm (Davies and Breslin, 2003a). All biosecurity programmes should be supplemented by appropriate monitoring to confirm their effectiveness.

Despite the difficulties in maintaining biosecurity, there appears to be little *Salmonella* infection on free-range layer and broiler farms (Davies and Breslin, 2003b; McDowell, 2004), so high levels of biosecurity do not appear to be necessary to limit *Salmonella* infection in less intensive systems.

5.6.2 Decontamination of contaminated premises

Feedmill

Persistent contamination of oilseed plants, feedmills, hatcheries and poultry farms is an important aspect of the epidemiology of *Salmonella*. Contamination of feedmills will be considered in more detail in Chapter 7 but the main focus of long-term contamination is normally the coolers (Davies and Hinton, 2000), from whence recycling can occur via contaminated dust, reworked material and residual contamination in bins and auger systems. Cooler contamination appears to be particularly problematic in integrated poultry companies, because there is insufficient time to close the mill and complete effective decontamination by cleaning, fumigation and multiple passage of formaldehyde or acid-treated material, such as wheatfeed, whilst maintaining a feed supply for company flocks. Where cooler contamination does exist, the salmonellas present are usually the predominant non-invasive serovars found in live poultry and at slaughter. Waves of broiler-flock infection can also occur when there is more transient contamination of mills.

Hatchery

A similar situation exists in hatcheries, where resident *Salmonella* serovars may become established, particularly in hatcher incubators, but, also, occasionally, in setters and tray-wash machines. Again, time constraints are the major problem, as setters may be occupied continuously and hatchers, the main site of contamination, are usually only empty for a few hours before re-stocking. This allows only limited time for effective disinfection and, if *Salmonella* is present, to eliminate it. In practice, it is necessary to carry out a very thorough clean, using noxious chemicals at high concentrations (Mitchell and Waltman, 2003) to overcome the problem. Disinfectants such as formaldehyde-glutaraldehyde-quaternary ammonium compound (QAC) combinations or more refined non-coal-tar phenols are best for disinfection of surfaces and ducting. When Salmonella is present, these should be used at DEFRA General Orders concentrations rather than general application rates, and should be applied to surfaces that have dried after cleaning and power washing with a QAC disinfectant solution. Trays of undiluted formalin should be used throughout the hatch, so that an inhibitory atmosphere is maintained for the whole of the time. This can be handled safely if fans are used to remove the vapour at take-off. Following successful completion of this programme, confirmed by testing, it will be possible to revert to less intensive disinfection, but care must be taken to avoid re-contamination from other parts of the building, such as waste-handling areas, and from infected breeding flocks. If infection in a breeding flock does occur, then attempts should be made to channel eggs through a limited range of incubators and handle eggs and chicks separately from those of other flocks. It is also necessary to ensure that there is minimal opportunity for cross contamination via air, equipment or staff movements (Cox *et al.*, 1990; Davies and Wray, 1994; Davies *et al.*, 2001).

Poultry farms

Cleaning and disinfection of premises for primary breeding and grandparent flocks is normally extremely good and well monitored to ensure that *Salmonella* does not gain access on the rare occasions when biosecurity breaks down. Persistent infection of parent chicken flocks used to be a significant problem in the UK, but improvements in cleaning programmes and rodent control, plus the use of vaccination for *S.* Enteritidis, have changed the situation completely, so persistence of infection is now extremely rare (Davies *et al.*, 2003). The situation in parent turkey and duck flocks is less clear, since there is no statutory monitoring, but results of voluntary monitoring suggest that persistence of *Salmonella* is not a major problem in these sectors.

Persistent infection of commercial broiler, turkey and duck farms is still a problem in some companies (Crilly *et al.*, 2001) but, overall, the standard of cleaning and disinfection has improved, so this is no longer so important. There are, however, problem farms in many companies and these are often large, modern farms with metal houses that should be relatively easy to clean. Persistent contamination often involves serovars that have originated from persistently contaminated feedmills, so these strains may have become environmentally adapted. There is no evidence of disinfectant resistance among such serovars, but, where they are present, it is necessary to allow more time between flocks for a thorough clean-out of all houses, before disinfection is begun. Attention to detail is vital to ensure that there are no residual foci of contamination (Engvall, 1993; Meroz and Samberg, 1995) and this can be done systematically to predict likely disinfection failures (Cruickshank, 2003; Rose *et al.*, 2003). Physical auditing, rather than checking procedures on paper, is essential to ensure that control measures and HACCP principles are applied properly (Noordhuizen and Frankena, 1999). As with the hatchery, it is necessary to upgrade disinfection until the persistent infection has been overcome, and similar disinfectants should be used, with the longest possible drying period between washing and disinfection and between disinfection and fogging with neat formalin solution. In large, modern houses, the high number of individual pan-feeders that accumulate wash-water is a particular problem, made worse by operator fatigue. Before re-stocking, feed and water lines should be completely emptied. Water lines should be flushed with an acidifier and, if feed lines are the problem, then acid or formaldehyde/acid-treated rations should be used at the end of the old flock and start of the new, to reduce *Salmonella* in the system.

Persistent contamination is more of a problem on commercial laying farms, particularly caged layer units, where *S.* Enteritidis may occur, despite vaccination (Davies and Breslin, 2003b). The cause of this problem is multifactorial, but, in general, it is difficult to clean and disinfect cages and droppings pits effectively and mice, rats and flies are a hazard (Davies and Breslin, 2003a). However, resistance of *Salmonella* to disinfectants is not involved (Davison *et al.*, 2003). The main weaknesses are the linkage of houses by egg and droppings belts and the close proximity of houses, which may allow dust to transfer *Salmonella* between flocks. In Denmark, where vaccination is not allowed, a

strict monitoring and control policy, including heat treatment of eggs from infected flocks, has forced producers to improve procedures. Disinfection of persistently contaminated houses with 30 ppm formaldehyde in steam at = 60°C, 100% RH has been introduced to overcome previous failures of disinfection (Gradel *et al.*, 2003, 2004). For many other countries, monitoring is insufficient to detect a large proportion of infected flocks, and this makes effective control very difficult (Muller and Korber, 1992). It has been shown experimentally that serial passage of *S.* Enteritidis through birds increases the rate of internal infection of eggs (Gast *et al.*, 2003), and there is concern that persistent infection of farms may lead to the selection of more invasive strains and organisms more likely to evade the immune response provided by vaccination. Ideally, total depopulation of persistently infected farms and an all-in/all-out production system should be used. Although this would be possible theoretically within a large company, since different sites could supply different grades of eggs, the downtime involved in the transition period would present severe economic difficulties (Bender and Ebel, 1992). Control of mice is also difficult once large breeding populations have become established, but, without the intensive baiting, trapping and tidiness necessary to achieve control, cleaning and disinfection of the premises is wasted. In the USA, Denmark and UK, improved control of egg contamination has led to a significant reduction in human cases of *S.* Enteritidis (Patrick *et al.*, 2004).

5.6.3 Vaccination

The cost of attempting *Salmonella*-free production has prompted the development of biological options, but rarely are these fully effective on their own (Barrow *et al.*, 2003). Use of vaccination for *Salmonella* control varies in the different production systems and among EU Member States. In Germany, it is compulsory to vaccinate laying flocks against *S.* Enteritidis, but this may be done with a *S.* Typhimurium vaccine that has limited efficacy against *S.* Enteritidis. In the Nordic countries, no vaccination is permitted at any level of production. Vaccination against *S.* Enteritidis is not used at the level of elite and grandparent flocks in the UK, but is almost universally used routinely in broiler parent flocks. Here, vaccination involves a killed *S.* Enteritidis/*S.* Typhimurium vaccine, a live *S.* Enteritidis oral vaccine or an oral and injectable *S.* Gallinarum 9R vaccine. In layer, turkey and duck parent flocks, targeted vaccination may be used following identification of a specific infection problem, to provide extra protection for birds placed in previously contaminated houses.

No vaccination is used for commercial meat birds, but it is thought that the stimulation of maternal immunity by vaccination of broiler breeders, using an injectable vaccine, may have contributed to the current rarity of *S.* Enteritidis in UK broilers. Certainly, maternal immunity can interfere with the colonisation of birds by a live *S.* Enteritidis vaccine strain, so the vaccine response will be less effective (Methner *et al.*, 2002). Vaccination against *S.* Enteritidis is a requirement for membership of the main quality assurance scheme used by

the egg industry in the UK and, since 2001, a live, oral vaccine has been used most commonly. Vaccination alone is not successful in caged laying flocks and must be combined with improved cleaning, disinfection and pest control (Davies and Breslin, 2003b, 2004a). There are constant debates about the relative efficacy of killed and live vaccines and, generally, live vaccines are considered to be superior (Zhang-Barber *et al.*, 1999), but fears about the safety and environmental persistence of the vaccine strains (Selbitz, 2001) may have led to the development of commercial vaccines that are considerably attenuated and therefore less robust than necessary for a high level of colonisation and stimulation of immunity. Also, there have been difficulties, in some cases, with consistent delivery of vaccine through long water lines and residual antibacterial substances in the water, eggs or chicks, following a previous flock treatment, that may affect the viability of the vaccine. More work is needed to develop and properly define the immune response to rationally-derived live vaccines (Mastroeni *et al.*, 2000) and application methods. For example, when tested under experimental conditions, competitive exclusion treatment and vaccination can give an additive response (Methner *et al.*, 2001) but, in practice, the competitive exclusion product may be given too long after exposure to *Salmonella* to be of value, especially in laying flocks.

In the future, improved vaccine delivery systems that are specific to poultry flocks are required (Tacket and Mason, 1999; Liu *et al.*, 2001; Ryan *et al.*, 2001; Oshop *et al.*, 2002; Mastroeni and Ménager, 2003; Singh and O'Hagan, 2003), as well as the ability to deliver protection against a wider range of *Salmonella* serovars.

5.6.4 Competitive exclusion

In some countries, the application of competitive exclusion products, which are undefined or partially-defined cultures derived from the intestinal microflora of poultry, has been widely used as a part of general *Salmonella* control programmes. Elsewhere, they may be used only within certain companies or for problem farms (Mead, 2000; Ricke, 2003). Currently, there are problems with the use of undefined competitive exclusion cultures in some Member States, because of variations in the authorisation procedures. The European Commission plans to consider this topic further, since such products can be valuable aids in the control of *Salmonella* and other pathogens.

Many different treatment products are available commercially and these appear to have different levels of efficacy (Nakamura *et al.*, 2002; Ferreira *et al.*, 2003). The efficacy is also related to the level of challenge, but, even when this is high, there is still, usually, some reduction in the prevalence of infection in individual birds and in the numbers of *Salmonella* organisms being excreted. The effect can be used to sequentially reduce the level of excretion and environmental challenge in consecutive flocks, to the point where total elimination is more likely (Mead, 2000). Wider studies are needed to fully define this phenomenon and further developments are in progress (Andreatti *et*

al., 2003). To be maximally effective, competitive exclusion treatment should be administered shortly before exposure to *Salmonella*, so administration by spraying chicks at the hatchery is generally superior to dosing on the farm via drinking water (Cox *et al.*, 1992; Mead, 2000; Patterson and Burkholder, 2003).

5.6.5 Feed and water treatments

A wide range of feed and water additives for the control of *Salmonella* are described (Ricke, 2003), but most require more large-scale field evaluation (van Immerseel *et al.*, 2002). In-feed preparations of organic acids can reduce the chances of flock infection, both from contaminated feed and environmental challenge (Humphrey and Lanning, 1988; de Oliveira *et al.*, 2000), but the efficacy of different products varies (Hume *et al.*, 1993) and those containing the highest levels of free formic acid in a liquid application appear to perform best.

A combination of formaldehyde and propionic acid appears to be helpful in reducing the shedding of *Salmonella* and environmental contamination in infected laying flocks, where the level of environmental challenge is not overwhelming (Davies, R.H., unpublished data; Anderson *et al.*, 2001, 2002).

Water can be a means of introducing *Salmonella*, so only mains water or water from other sources that has been appropriately treated and tested should be used. Treatment of water supplies with acidic oxidising agents, such as hydrogen peroxide/peracetic acid or lactic acid (Byrd *et al.*, 2001) or sodium chlorate and sodium nitrate (Byrd *et al.*, 2003; Jung *et al.*, 2003) appears to have a beneficial effect on broiler contamination at slaughter, and could be investigated in a wider range of situations. It is possible to use continuous water and air disinfection during the life of a flock by means of organic acid or chlorine-based products (Tablante *et al.*, 2002; Bragg and Plumstead, 2003). Lime can also be used to achieve a high pH and reduce pathogens in litter and around entrances to houses (Bennett *et al.*, 2003; van Immerseel *et al.*, 2004).

5.6.6 Antimicrobials

Antimicrobials are primarily used in poultry for prevention or treatment of disease (ACMSF, 1999), but they may also be used to restrict the spread of *Salmonella*, reduce the chance of flock infection where there is an environmental challenge, or, in some countries, reduce the prevalence of contamination prior to slaughter. Treatment with fluoroquinolones, followed by competitive exclusion treatment has been used in the control of *S.*Enteritidis in broiler-breeder flocks (Goren, 1993; Reynolds *et al.*, 1997) and layer rearing flocks, but is seldom completely effective (Humbert *et al.*, 1997; Davies *et al.*, 2003). Treatment of eggs and chicks with antimicrobials, such as fluoroquinolones, cephalosporins or gentamicin, has also been used to limit vertical transmission (Hafez *et al.*, 1995), but may interfere with other control measures, such as use of live vaccines or competitive exclusion cultures (McReynolds *et al.*, 2000). Prophylactic or therapeutic use of antimicrobials, such as ampicillin, tetra-

cyclines or spectinomycin, may also suppress *Salmonella*, but, in the case of strains with multiple resistance, e.g. *S.* Typhimurium DT104, use of these compounds may increase the multiplication and spread of the organisms. In recent years, there has been concern about treatment failures involving *Salmonella* and *E. coli* with reduced susceptibility to ciprofloxacin. It is unclear exactly what proportion of the overall problem has been generated by veterinary usage, but there is a temporal association between increasing usage in poultry and reduction of susceptibility in human isolates (Bager and Helmuth, 2001; Malorny *et al.*, 2003). Reduced susceptibility to fluoroquinolones appears to have been a particular feature of *Salmonella* isolates from turkeys (Davies *et al.*, 1999), but following the introduction of prudent-use initiatives, the prevalence of such strains in UK poultry production has fallen (VLA, 2003). Despite the reduction in susceptibility to fluoroquinolones, high-level resistance (i.e. MICs > 1.0 mg/l) is rarely seen. This may be due to reduced survival and dissemination capabilities ('fitness') in the strains (Giraud *et al.*, 2003). There is also concern about increasing multiple resistance in *Salmonella* (Jones *et al.*, 2002), and strains such as *S.* Paratyphi B var. Java have spread widely in some countries (van Duijkeren *et al.*, 2003). There is further concern about the possible spread of plasmid-mediated resistance to third generation cephalosporins, such as ceftriaxone (Liebana *et al.*, 2004), amongst *Salmonella* and *E. coli* via international trade. The reduction in use of antimicrobial growth promoters, that had either no effect or some suppressive effect on *Salmonella* (Cox *et al.*, 2003), and consequent increase in the use of therapeutic antimicrobials, may increase the potential for selection and spread of resistant organisms.

5.7 Control of *Campylobacter*

5.7.1 General considerations

Options for control of *Campylobacter* are less well defined than those for *Salmonella*. Feed and vertical/hatchery transmission do not have a major role, if any, in dissemination of infection. Persistence of the organism within depopulated poultry houses is occasionally possible (Petersen and Wedderkopp, 2001), especially when short turnaround times allow pooling of wash-water or when water systems are subject to contamination, but it is unlikely that cleaning and disinfection programmes that are sufficient to control *Salmonella* would fail to eliminate *Campylobacter*. Prolonged survival in dry materials in inaccessible areas of the house, which is a feature of *Salmonella* contamination, does not occur regularly (Berndtson *et al.*, 1996b), but may be underestimated in the short term, because of the limitations of present culture methods (Cox *et al.*, 2001). *Campylobacter* is widespread in domestic animals, wildlife and aquatic environments (Newell, 1999) and, in many ways, acts as a commensal or opportunist organism (Humphrey, 1989). In infected flocks, the organism achieves high levels in intestinal contents, so personnel and equipment that have been in contact with the birds will be highly contaminated in the short term.

Viable *Campylobacter* can also be found in dust emanating from houses that hold infected flocks, although the infectivity of the organism in this dry material is unknown. Because of the large potential challenge, it is common for all houses on a multi-house site to become infected. However, spread can be limited by good biosecurity, suggesting that airborne infection is not a major route.

Having eliminated a number of potential sources from consideration, as discussed above, the environment immediately surrounding the house is left as the predominant source of infection. This apparently simple situation is made complex through the variety of potential routes by which contaminated material can be brought into the house (White *et al.*, 1997; Sahin *et al.*, 2002). Despite the apparently straightforward concept of preventing organisms entering the house, hygiene barriers that have been effective for *Salmonella* have had only limited success with *Campylobacter* (Kapperud *et al.*, 1993; Humphrey *et al.*, 1993; Berndtson *et al.*, 1996a; Reiersen *et al.*, 2001). This is probably because of the relatively high numbers of organisms involved and the low infectious dose. Intervention studies have shown a reduction in *Campylobacter* or a delay in establishing infection. Gibbens *et al.* (2001) showed a 50% reduction in the risk of flock infection and identified lack of water sanitisation as the most significant risk factor. In Norway, only 6.3% of 3267 flocks were found to be infected, and this favourable situation is thought to be related to a stricter attitude to the maintenance of hygiene barriers (Hofshagen and Kruse, 2003). In Norway, however, farms are smaller, often with only one house on the site; they are more likely to be run by the owner and the climate is colder. A substantial reduction in flock prevalence has been achieved in Iceland, and the beneficial effect on human cases has been further enhanced by freezing broiler carcasss (Stern *et al.*, 2003).

Observations by the author on UK broiler farms suggest that, despite apparently good company protocols, biosecurity precautions on entry to the house are often not properly observed and the hygiene barrier area becomes contaminated with faecal material, so cannot operate as intended. This is especially problematic on farms where disinfectant foot-dips are no longer used. In addition, proper hand sanitisation is not carried out regularly and poorly dried hands that are not disinfected may present an increased risk. Furthermore, moveable equipment may not always be properly disinfected between houses or even between sites. In summer, when there may be more organisms around the house as a result of wild-bird and insect movements, and contaminated water supplies, standards tend to fall further, as farm staff do not always use waterproof boots or wear other protective clothing. One study has shown that a high risk associated with children entering poultry houses was reduced when they used house-dedicated clothing (Bouwknegt *et al.*, 2004).

It is apparent that attention to detail is needed at all times (Pattison, 2001) and, in broiler-parent rearing flocks, this can keep birds *Campylobacter*-free until they are moved to laying farms, when control becomes impossible (K. Gooderham, personal communication). Rigorous attention to detail is laborious and therefore costly. Thus, it is necessary to provide sufficient incentive to stimulate compliance, and a premium of one penny per bird for flocks that were

negative at slaughter would encourage better hygiene and an improved approach to flock thinning. It is also desirable to develop boots and other protective clothing that are light, cool and pleasant to wear, and can be changed easily and rapidly by farm staff and thinning teams. Thinning would be less of a risk, if washed modules and crates were immersed briefly in a 2–3% solution of a compound oxidising disinfectant, but there is still a chance that contaminants from vehicles, catching gangs and the exterior of the house will be brought in during thinning. It would be desirable, therefore, to devise a barrier system that would allow the remaining birds to be held back from the area where thinned birds had been removed until the following day, when any *Campylobacter* cells introduced would have died off. Even if *Campylobacter* is introduced at thinning, the numbers carried into the abattoir will typically be lower than for flocks infected at an earlier stage and this will reduce the number of human infectious doses per flock (Hartnett *et al.*, 2001).

Since there is a clear relationship between *Campylobacter* and water, it makes sense to ensure that the water supply is as safe as possible. Many farms use bore-hole water, which may be subject to contamination after heavy rain. Even if chlorinated mains water is used, the level of chlorine at the end of a long water line may have dropped significantly. Therefore, it makes sense to use water sanitisers, at least intermittently and strategically, during periods of stress, such as chick placement and dietary changes, at the end of the 'lag phase' and during thinning. Water acidifiers often improve the performance of flocks (Tablante *et al.*, 2002), although there may be differences between products and possible damaging effects on water lines. Silver-stabilised acid products appear to be superior in this respect and other substances, such as lime, aluminium sulphate or sodium bisulphite, may be useful to reduce numbers of organisms in litter, possibly after thinning of a partitioned area of the house (Line, 2002).

Since *Campylobacter* does not colonise abattoir equipment or multiply in the environment or on carcasses, testing and logistic slaughter of flocks would have only a minor effect on the total burden of contamination entering the food chain (Rosenquist *et al.*, 2003). However, it would be desirable for flocks that are inevitably contaminated, such as organic flocks (Heuer *et al.*, 2001), to be slaughtered only at the end of the day. If a two-log reduction in the number of organisms per carcass could be achieved this would have a substantial effect on the level of human disease (Stern and Robach, 2003). Methods of achieving a reduction in numbers in infected flocks prior to slaughter should be evaluated. These could include the use of water or feed acidifiers, other harmless inhibitors, bacteriocins and bacteriophage, possibly combined with competitive exclusion products (Stern, 1994).

5.7.2 Antimicrobial resistance in *Campylobacter*

Although antimicrobials are never used to control *Campylobacter* in poultry flocks, the high prevalence of infection means that these organisms will often be exposed to whatever treatment is given for clinical disease or prophylaxis.

Campylobacter responds very quickly to the use of antimicrobials by developing resistance (Avrain *et al.*, 2003), especially to fluoroquinolones (Ge *et al.*, 2003; van Boven *et al.*, 2003). Resistance is more frequent in situations where there is high use of fluoroquinolones, e.g. in developing countries or in turkeys (Ge *et al.*, 2003; Padungton and Kaneene, 2003), but, unlike *Salmonella*, there appears to be little spread of specific, resistant clones (McDermott *et al.*, 2002).

5.8 Future trends

In the near future, there will be an increasing requirement for monitoring, target-setting and control of *Salmonella* that will extend to broilers, commercial layers and turkeys, as laid down in EU Zoonoses directives (EC 2003a,b). Such target-setting will strengthen the case for wider harmonisation of sampling and test methods. In the UK, considerable progress has been made in reducing *Salmonella* contamination of broiler meat and eggs (FSA, 2004), with most contamination of eggs being only on shells. This situation may be improved further by new egg-washing procedures, now permitted in the EU (EC 2003c).

In the free market, international trade in poultry breeding stock remains a threat (Ashton, 1984; Vågsholm, 1998), as shown by the consequences of importing layer hatching eggs contaminated with *S.*Enteritidis PT4 in 2003. Imported chicken and eggs are used in catering, because of their lower cost (Cowden *et al.*, 2003). *Salmonella* Paratyphi B var Java, which has spread explosively and become persistent in broiler production in the Netherlands, Belgium and Germany (van Pelt *et al.*, 2003), has multiple resistance to antimicrobials and has been found in imported poultry meat and human disease associated with such meat in the UK (Brown *et al.*, 2003). Rapid confirmation of *S.*Paratyphi B var Java can be difficult, so improved tests are being developed for detection and fingerprinting (Malorny *et al.*, 2003; Prager *et al.*, 2003). Such strains with multiple resistance may also introduce new resistance mechanisms, which could spread subsequently to other serovars (van Duijkeren *et al.*, 2003).

There are similar threats from further afield. The Far East has been suggested as a significant source of mobile, antimicrobial-resistance elements, probably because of the close association between poultry, humans and aquaculture, and a favourable climate for the development and spread of resistance in the environment, as well as less stringent control of the use of antimicrobials (Teale, 2003). As epidemic strains of *Salmonella*, such as *S.* Enteritidis and *S.* Typhimurium DT104, come under control or subside naturally, there are fears of new epidemic strains being created by phage, plasmid or integron transfer (Canchaya *et al.*, 2003). One such candidate strain with multiple resistance is AmpC *Salmonella* Newport, which has emerged in the USA, primarily in cattle and humans, but with some cases in poultry (Zhao *et al.*, 2003). This strain has already caused an outbreak in humans consuming contaminated horsemeat imported from the USA into France, but does not appear to have spread yet into European livestock. Improved surveillance to identify new strains with epidemic potential will

become more important in future, and tests, such as microarrays, which can determine multiple genes simultaneously, will become incorporated into surveillance programmes, as the cost falls. There is the potential for these methods to completely replace phenotypic typing, such as serotyping and phage typing. More discriminatory tests will allow improved tracking and assessment of sources, e.g. the various poultry species or eggs, red meat, environment, etc., which have been long-standing requirements in order to prioritise further controls (Humphrey *et al.*, 1988). Also, to assist prioritisation, more use will be made of quantitative risk assessment models (Vose, 1998; Nauta *et al.*, 2000), which will require more quantitative data, creating a need for more efficient, less costly enumeration techniques (Chen *et al.*, 2003). The models themselves should also be evolving continuously, rather than being based on the life-span of a research project, before being scrapped. Integration of QRA modelling into long-term surveillance would facilitate such development and the filling of data gaps.

In parallel with ongoing improvements in biosecurity and husbandry aspects, such as diet and stocking rates, there is likely to be work on improving vaccines and delivery systems (Tacket and Mason, 1999; Ryan *et al.*, 2001; Oshop *et al.*, 2002; Barrow *et al.*, 2003). Improved gene-selection techniques will also hasten the development of strains of poultry with enhanced genetic resistance to infection (Barrow *et al.*, 2003; Kramer *et al.*, 2003a,b). There is also likely to be increasing regulation of waste from poultry farms and other sources, because of environmental pollution considerations, while appropriate investment in methods like controlled composting will see such material made safe (Henuk and Dingle, 2003).

Similar considerations apply also to *Campylobacter*, but more work is required to prioritise the potential sources of flock infection, so that controls can be better targeted. Better intervention studies are also required, so that the effectiveness of present options, such as biosecurity and water treatment, can be assessed properly, both in situations where they can be applied rigorously on controlled, model farms and more widely under field conditions to identify precisely the practical barriers to implementation. The proposed intervention studies should be large enough to identify the effects of individual interventions, such as water treatment by, for example, electrolytic oxidation (Wilhelmson, 2003), hand washing, etc., and to investigate their efficacy in appropriate combinations.

Work will continue on biological control options. Phage treatment has been proposed to reduce the intestinal burden of *Campylobacter* immediately before slaughter, and strains of *Campylobacter* that are apathogenic for humans may be used for vaccination/competitive exclusion (Widders *et al.*, 1996; Newell and Fearnley, 2003). Improved methods for enumerating *Campylobacter* are also needed and harmonisation of typing methods is required for routine surveillance and epidemiological investigations. There appear to be opportunities for investigation of preventive method/bird-strain combinations (Laisney *et al.*, 2004). The relevance of poultry as a possible source of other, related organisms, such as *Arcobacter butzleri* (Eifert *et al.*, 2003), will also become clearer in future years.

5.9 Sources of further information and advice

5.9.1 *Salmonella*

Association of Official Analytical Chemists (international, particularly USA, methods and information exchange) (www.aoac.org)

British Egg Industry Information Service (BEIS) (www.britegg.co.uk)

British Poultry Council (BPC) (represents poultry meat sector) (www.poultry.uk.com)

British Veterinary Poultry Association (BVPA) (www.bvpa.org.uk)

Communicable Disease Report Weekly. Published by Health Protection Agency, Colindale, London

EU Annual Report on Trends and Sources of Zoonotic Agents in the European Union and Norway. (Also includes *Campylobacter* and other pathogens.)

European Food Safety Authority (EFSA) (scientific opinions, regulations, etc.) (www.efsa.eu.int)

International Organisation for Standardisation (ISO) (test methods, validation procedures) (www.iso.ch)

Office International des Epizooties (annual reports, manual of test standards) (www.oie.int)

PulseNet (harmonised molecular typing network) (www.aphl.org)

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Salmonella Community Reference Laboratory (newsletters, publications and workshops) (www.rivm.nl/crlsalmonella)

Salmonella in Livestock Production in GB. Published by Veterinary Laboratories Agency-Weybridge, Addlestone, Surrey KT15 3NB.

Scottish Centre for Infection and Environmental Health Weekly Report. Clifton House, Clifton Place, Glasgow G3 7LN.

UK Department for Environment, Food and Rural Affairs (national legislation, codes of practice, research programmes) (www.defra.gov.uk)

UK Food Standards Agency (information sheets, strategies, research programmes) (www.food.gov.uk)

US Communicable Disease Centre (www.cdc.gov)

US Department of Agriculture Information Service (www.fsis.usda.gov)

US National Antimicrobial Resistance Monitoring System (www.cdc.gov/narms/)

World Health Organisation Collaborating Centre for Reference and Research on *Salmonella* (www.pasteur.fr)

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5.9.2 *Campylobacter*

There are more limited specific sources of information on *Campylobacter*. Recent reviews and scientific papers provide the best material. A number of the sources listed under *Salmonella* also consider *Campylobacter*.

CAMPYNET (network of harmonisation of molecular typing) (http://campynet.vetinst.dk)
Eurosurveillance (reports of disease outbreaks, etc.) (www.eurosurveillance.org)
US Agricultural Research Service (research and control strategies) (www.ars.usda.gov)

5.9.3 General textbooks

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6

Catching, transporting and lairage of live poultry

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6.1 Introduction

Observing birds being shackled at the processing plant will indicate that there are great differences in cleanliness between batches after catching, transport and lairage. It is logical to assume that the more faecal material there is on the skin and feathers, the faster and greater will be the contamination of both processing plant and carcasses by enteric organisms. Stern and Robach (2003) found that levels of *Campylobacter* at the production and processing stages were not strongly correlated and they suggested that this 'indicated the existence of complex parameters involving production factors and variables associated with flock transport and the processing of the broilers'. In an earlier study, Stern *et al.* (1995) showed that levels of *Campylobacter* on the carcasses were significantly raised from 12.1% harbouring an average of 2.71 log cfu per carcass before transport to 56.0% averaging 5.15 log cfu per carcass after transport. This showed that transport and lairage contributed to the numbers of *Campylobacter* normally found on processed carcasses.

Decontamination, whether of carcasses or equipment, is generally considered to remove only a proportion of the microbial contaminants present, so that the lower the faecal contamination of catching and transport equipment and the cleaner the birds, the greater the chance of keeping enteric pathogens at low levels in the processing plant and on the final, packaged product.

It is clear that poor biosecurity, such as inadequate cleaning of the transport systems before re-use and the spread of contamination from farm to farm by catching crews, will greatly increase the risk of cross-contamination between flocks. A few studies have been undertaken to investigate the risk of contaminating

clean broiler flocks with salmonellas and campylobacters during catching and transport. For example, Slader *et al.* (2002) found both types of organism on the feathers of previously negative birds, following the transport process. Analysis of the results obtained in this study showed that catching and placing the birds in crates significantly increased their chances of being contaminated with *Campylobacter*, while the risk of contamination with *Salmonella* was also significantly greater after the birds had been handled by the catchers. The transport stage is particularly important, since birds can spend considerable periods of time in the transport containers during loading, transport, lairage and shackling. Warriss *et al*. (1990) found that journeys could be up to 12 hours in the UK.

Another factor, often mentioned, but not thoroughly investigated, is the increase in shedding of pathogens by the birds, mainly onto the transport equipment. Stress, whether emotional or physiological, is generally considered to have a bearing upon this increase in shedding (Mulder, 1995). A study by Whyte *et al*. (2001) demonstrated that transport-induced stress increased shedding of *Campylobacter* via faecal material, although distance and duration of the journey had no significant impact. Mengert and Fehlhaber (1996) investigated stress in the live bird and subsequent endogenic contamination. They compared normal transportation, a 48-hour rest period and transport with additional heat-stress on the broilers. Normal transportation caused a contamination rate of nearly 50%, which was not affected by heat stress. However 10% of the birds suffered from a bacteraemia induced by the stress. It was concluded that stress aggravates the microbial risk in relation to the end product.

The faeces produced by the birds can vary in volume and consistency, depending on the regime used for feed withdrawal, and this may influence bird cleanliness. In particular, Wilkins *et al.* (2003) found that most birds were dirty, with cleanliness scores of six or more (on a scale of 0–8, with eight as the dirtiest). Nevertheless, some individual loads were much cleaner, although it was not possible to determine what influenced the cleanliness score.

A possible route of flock infection is via environmental sources. Studies on farms have shown that *Campylobacter* can be found in puddles, on roadways and even in the air downwind of rearing-house extractor fans (Allen *et al.*, 2003). Following some controlled experiments, Nakamura (1997) suggested that horizontal transmission of *Salmonella enteritidis* could be affected by patterns of air flow.

In conclusion, there are several routes of contamination and cross-contamination that could impact on the hygiene status of the live bird during pre-slaughter handling. In this chapter, contamination factors are discussed and possible improvements to catching, transport and lairage systems in relation to food safety are considered.

6.2 Improving catching and transport systems

The hygiene hazards that arise during catching and transport of birds are largely due to breakdowns in biosecurity measures and possibly certain aspects of feed

withdrawal and bird stress, leading to greater shedding of pathogenic organisms. During depopulation of the farm, the main biosecurity measures that are in place for the rearing of housed flocks are usually breached. Prior to removing the birds, the main doors are normally opened and hygiene barriers or disinfectant foot-dips are no longer used, enabling contamination from pests or debris from the surrounding environment to enter the house. At the same time, contaminated material from a positive flock can be disseminated throughout the farm. Measures such as foot-dipping and changing of footwear between houses have been shown to either delay or prevent colonisation of flocks with *Campylobacter* (Humphrey *et al*., 1993; van de Giessen *et al*., 1996). The catching crew may have collected birds previously from several farms and frequently vehicles or forklift trucks are not disinfected between these visits. In addition, the items in question are extremely difficult to clean and disinfect effectively. Handling of crates and placing modules either in the area in front of the doors or inside the house pose a risk, since either crates or modules can contain material from previous flocks or farms. Mechanised catching systems have also been identified as a source of *Salmonella* contamination (Ingmer, 2004), but, as discussed later, there are potential advantages to improving biosecurity by using a mechanical harvester.

A particular practice, with major implications for flock biosecurity, is partial depopulation or 'thinning', where a proportion of the flock is removed for slaughter before the normal age. This is practised by the majority of companies in the UK and it reduces bird stocking density, while allowing higher initial densities and thereby providing greater productivity, as well as a range of carcass weights. However, epidemiological studies have indicated that the process of thinning is a major risk factor for introducing infection into the broiler house (Evans, 1997; Evans and Sayers, 2000; Hald *et al*., 2000). It is difficult to maintain good biosecurity when the catchers brought in are expected to work rapidly before moving themselves and their equipment to another farm.

6.2.1 Improvements in biosecurity

Improvements at the catching stage have to be centred on achieving good biosecurity at all times, especially during flock thinning. A thorough risk analysis should include factors such as provision of made-up roads and hardstanding, so that any disinfected equipment, such as forklift trucks, transport modules and lorries, can be kept clean. There should be effective disinfection systems for the vehicles and proper facilities for personnel to change clothing and footwear, whether arriving at the farm or moving between houses. These measures are well known, but others should be considered. Since campylobacters have been found in the air-stream issuing from an extractor (Allen *et al*., 2003), it is sensible to consider depopulating the houses in a manner that minimises the risk of wind-borne particles spreading contamination to other houses, including those that have been cleaned and disinfected for re-population.

6.2.2 Mechanical catchers

Mechanical catchers, also known as broiler harvesters, need to be thoroughly cleaned and disinfected between farms and houses, but do have a significant role in improving biosecurity. They only have between one and three operators and reduce greatly the number of potential lapses in biosecurity measures. Furthermore, the bird containers do not come into contact with the contaminated litter. In one type of machine, the transport module is mounted on a trailer that follows the mechanical catcher around the house. With another type of harvester, the transport module does not enter the house at all; instead, a self-propelled unit collects a few hundred birds, which are then off-loaded outside the house, into the transport system.

6.2.3 Minimising bird stress and injury

Two of the most stressful experiences for poultry are thought to be close contact with humans and being held in an inverted position. Duncan (1989) reported that the heart rate of the bird increased by about 70 beats per min, when the bird was approached by a human catcher, and 100 beats per min when lifted manually. If the bird was carried upright, the heart-rate started to fall, but, when inverted, the heart-rate remained high. Kannan and Mench (1997) showed that corticosterone levels in broilers were highest immediately after handling, but handling in the upright position was less stressful in this respect than keeping the birds inverted (Kannan and Mench, 1996, 1997). The design of broiler harvester (Berry *et al.*, 1990) ensures that injury and stress levels in the birds are kept low (Duncan *et al.*, 1986; Ekstrand, 1998). However, the impact of lower levels of stress at catching on shedding of pathogens and carcass hygiene is unknown. Gregory (1998) states that, when bruising occurs before slaughter, there is a risk that the bruise will carry *Staphylococcus aureus*. This indicates that, apart from bird-welfare and carcass-quality implications, there are also food safety consequences in avoiding stress and injury in the birds. Currently, the use of mechanical broiler harvesters aims to improve the welfare of both birds and operatives, while reducing levels of carcass downgrading. It can be anticipated that future designs will also ensure that they are easier to clean and use in a manner that facilitates good biosecurity.

6.2.4 Feed withdrawal

Feed should be withdrawn to allow the gut to become empty and therefore reduce the risk of extensive leakage of gut contents during evisceration. Obviously, feed withdrawal should not cause bird-welfare problems or unnecessary weight loss in carcasses (Gregory, 1998). Northcutt *et al*. (2003) investigated the use of a replacement finisher feed and length of feed-withdrawal period on carcass yield and microbial contamination. The study showed that counts of *Campylobacter*, coliforms and *E. coli* were unaffected by the type of feed or the period of feed withdrawal, either before or after evisceration. A study by Warriss *et al*. (2004) indicated that feed withdrawal may impact on the amount and consistency of the faeces and therefore on the cleanliness of the birds and transport containers.

6.2.5 Modular transport systems

Over the last few decades, modular transport systems have become the norm. These systems offer operational advantages and result in fewer injured birds, in comparison with individual crates and fixed-crate systems on lorries. The modular systems are demountable and can be cleaned in mechanised washers, as discussed later. In the UK, most birds are transported in the Anglia Autoflow system, using removable drawers. Twelve drawers are fitted into a module, which is then handled by a forklift truck. The drawers are made of high-density polypropylene, with extensive webbing to confer strength. There are two designs for the crate base: one with a closed floor, the other with an open floor (Fig. 6.1). The sides of both are highly perforated to allow air movement.

Open-floor crates are generally preferred because, according to anecdotal evidence, the birds are cleaner on arrival at the processing plant. Closed floors were thought to prevent excreta from birds in the upper tiers falling on those below. However, the birds became more soiled in their own excreta, possibly exacerbated by increased stress levels, when they were unable to grip the floor during vehicle movement. Another possible advantage of open floors is that air is not trapped beneath the inverted crates, when they are placed in the soaking tank during the washing process (see Section 6.3.2). When in use, the surface of the transport crate will become covered in a biofilm, a thin layer of adherent material that supports and protects microbes. The biofilm is notoriously difficult to remove by pressure washing, even from smooth surfaces. That present on drawers will occupy most of the surface and penetrate cracks, scratches and crevices that develop with use.

The modules in which the crates are housed are made from stainless or galvanised steel and have a skeletal structure that requires a special washer for

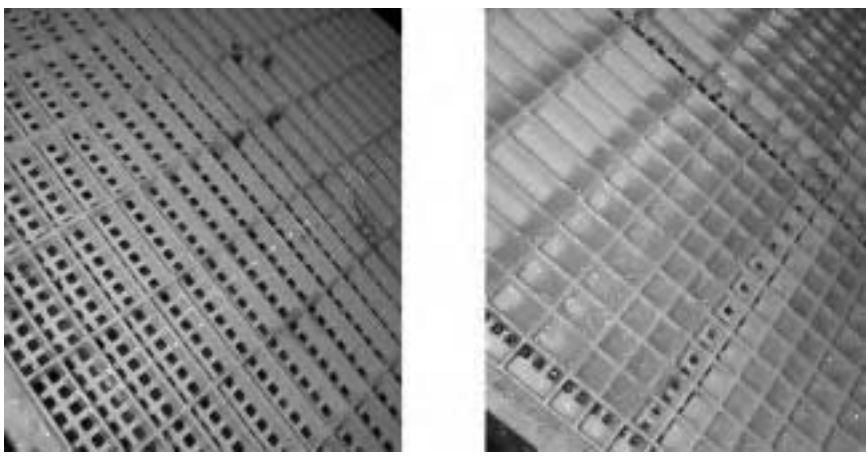

Fig. 6.1 Examples of open (grid-floor) and solid-floor crates (underside shown). Both types are in common use and there are those in favour of each. Higher Hygiene Assessment Scores can be awarded in the UK for factories using exclusively solid-floor crates, but they can be more difficult to wash, especially due to drainage problems.

adequate cleaning. When used in poultry houses, the modules can collect impacted litter, which is difficult to remove and so may represent a source of cross-contamination, especially during flock thinning.

Attempts have been made to reduce bird stress, whether due to cold or heat, by using controlled, mechanical ventilation in the transporter (Kettlewell and Mitchell, 2001). Anecdotal evidence indicates that broilers transported by these mechanically-ventilated vehicles are much cleaner, when they are shackled at the processing plant. It is not known whether this is an effect of lower stress levels, perhaps impacting on the amount and consistency of the faeces produced, or is due to drying of faeces by the action of the ventilation system.

6.3 Improving sanitation of transport systems

6.3.1 The need for improved drawer washing

There is a need to improve the cleaning of the individual components of the transport system at the processing plant, before the transporter is returned to a farm to collect more harvested birds. Transport crates are often contaminated with salmonellas and campylobacters, when they arrive at the farm, despite having been cleaned at the factory (Corry *et al.*, 2002). Berrang (2004) found that storing soiled transport crates for 48 hours did give a worthwhile reduction in *Campylobacter* contamination but, given the size and cost of the transport modules, it would not be practicable to increase the number available in order to put this strategy into practice.

Warriss *et al*. (2004) investigated the impact of feed and water withdrawal on the amount and nature of the gut content. They found that the maximum effect of deprivation on defaecation was seen after about four hours, while the amount of gut content was most affected after about 12 hours. With longer fasting, the gut content tended to become wetter, which could have an impact on bird cleanliness and cleaning treatments used for the transport system.

Studies have revealed that drawer-washing systems, as used in commercial processing plants, do not reliably remove *Salmonella* and *Campylobacter* (Humphrey and Allen, 2002). Two recent studies involved the UK poultry industry (Corry *et al*., 2002; Slader *et al*., 2002), while earlier work was carried out in Canada (Rigby *et al*., 1980a,b). Humphrey and Allen (2002) concluded that there had been little improvement in the hygiene of the process over the intervening period. The earlier work focused on *Salmonella*, while the more recent research in the UK investigated the role of the equipment in re-infecting broiler flocks with *Salmonella* and *Campylobacter*. Both sets of studies showed that more drawers were *Salmonella*-positive after being passed through a washing system than those examined before washing. Thus, the cleaning process was increasing the likelihood of cross-contamination, rather than serving as a control point.

Lister (2001) cites work by the Belgian Faculty of Veterinary Medicine and the European Union (EU) Food and Veterinary Services in Dublin showing that

Salmonella and *Campylobacter* could be isolated from poultry carried in contaminated transport drawers, even though the flocks were free from the organisms at the farm. Further work confirmed the findings of Rigby *et al*. (1980a) that the number of contaminated drawers increased after passing through a drawer-washing system. McKenna *et al*. (2001) examined the contamination of transport drawers and modules with *Campylobacter* and, at one of three plants, they also found that the number of contaminated drawers increased after passing through the washing process. At another plant, the washing process appeared to be very effective during the winter, with the incidence of *Campylobacter* falling to zero after washing, but results obtained in June showed that the contamination rate was as high after washing as it was beforehand; however, no explanation was given for this change.

Post-wash chemical disinfection should ensure low levels of microbial contamination, but Corry *et al*. (2002) found that the treatment could be ineffective, because, the disinfectant was applied at a low concentration and faecal soiling was still evident. Even after thorough application of disinfectant by hand, at the recommended concentration, drawers were not reliably free from *Salmonella*.

6.3.2 The existing drawer-washing process

The overall washing process

The general scheme for the modular system with separate, removable drawers is summarised in Fig. 6.2. This is the most common system in the UK, although other systems exist in which the drawers are permanently contained in the

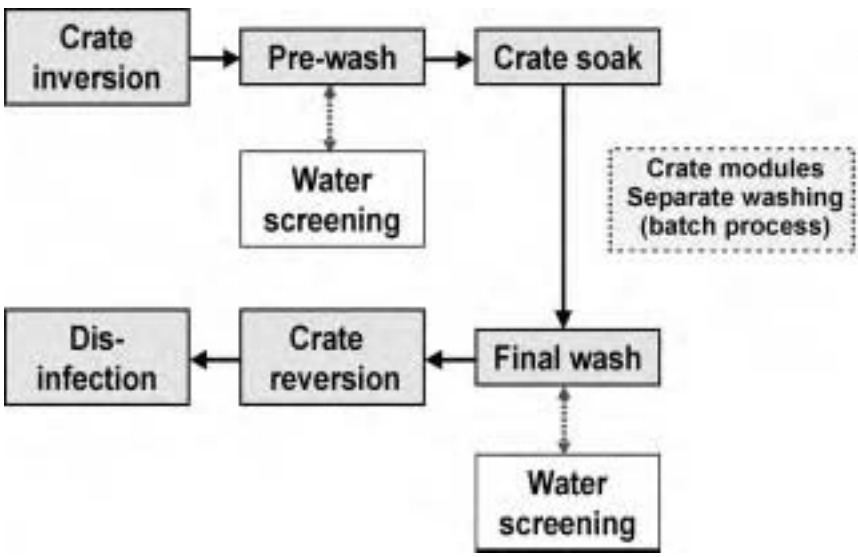

Fig. 6.2 Schematic layout of a typical drawer-washing process.

module and require a different washing system, but the general principles are similar. The process runs continuously during the processing period. At the end of production, the washing plant is drained and thoroughly cleaned with water. At present, the generally accepted target of the washing operation is to remove obvious debris to leave a 'visibly clean drawer', in line with general hygiene in the context of 'good housekeeping'. Microbiological objectives are not yet part of the washing operation.

Drawers are soiled by faeces, uric acid, litter material and feathers from the birds, and their rapid inversion during the cleaning process encourages loose debris to be removed. A pre-washing (Fig. 6.3) with cold-water sprays, using water that is usually recycled from the soak-tank, removes considerable amounts of the debris present. This water loop is often kept separate from the remainder of the washing process. A key part of the process is the soak-tank (Fig. 6.4), where attached debris may be softened. Two minutes of soaking is typical, but, in some cases, this can be longer in an effort to enhance the cleaning process.

Variations in the design and mode of operation include agitation of the soak-water to facilitate removal of debris on the drawer and the use of warm (25–50 ºC) or even hot (over 60 ºC) water. Addition of cleaning and disinfectant chemicals to the soak-tank is fairly commonplace, but the amounts used vary widely. The drawers can carry over as much as a litre of water into the main washing section, which is a second spray booth (Fig. 6.5). Water carry-over can

Fig. 6.3 Pre-wash booth and associated water recirculation equipment. The run-down screen that filters the water is just out of view at the bottom right of the photograph. Screened water collects in the main tank, from where it is recycled to the jets in the booth and to the soak tank. Make-up water is added to this tank.

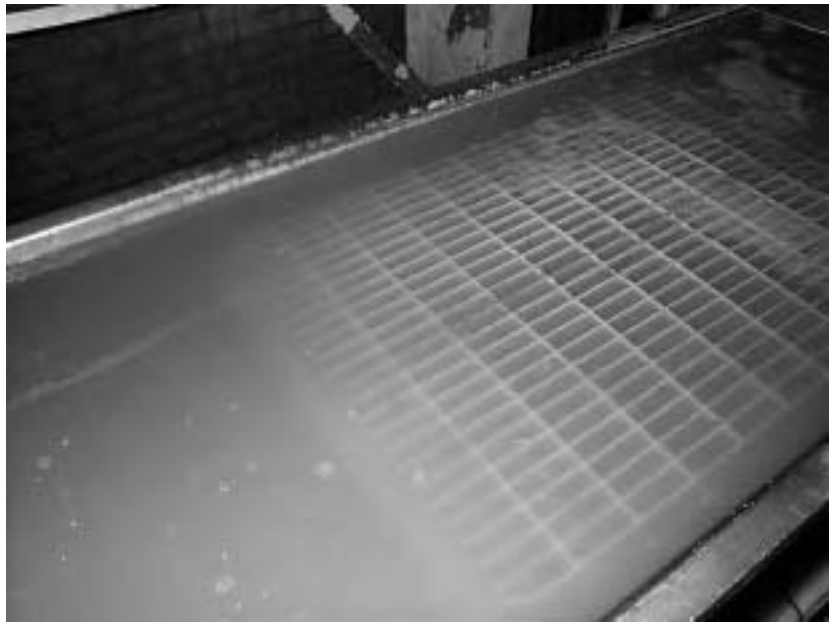

Fig. 6.4 The main soak tank with submerged drawers passing through. The length of these tanks can vary but, typically, they can hold between five and ten drawers. Plant speed will affect the actual residence time, which is normally around one minute.

Fig. 6.5 Drawers entering main wash booth. The run-down screen is visible in the lower part of the photograph; some fouling is evident.

also extend to the rinsing and disinfection stages. The main water jets again deliver recycled water, at pressure, over the various surfaces of the drawers. In some plants, the final rinse involves hot, clean water, but this is uncommon. If a disinfectant is used, it is applied via spray nozzles. A wide range of disinfection systems and chemicals are in use. Drawers are restored to the upright position and installed in the modules, which will have been washed separately, usually in an automatic spray booth.

Re-using wash water with screening
Most plants recycle wash water in order to minimise both effluent-disposal costs and supply costs for fresh water. The main treatment processes are (i) use of a run-down screen to remove debris and (ii) the addition of chemicals. Screens are usually part of the plant, but the addition of chemicals is often regarded as optional.

6.3.3 Improvement options for existing equipment

Normally, the drawers are dry prior to the pre-wash operation and the dwell-time in the latter is only 10–20 sec, so the full potential of the process may not be achieved. However, re-locating the pre-washer with the main washer to provide additional washing or rinsing could be a better way to remove the softened debris more thoroughly.

Clarification of the wash water is important, because nozzle blockages are a common problem at all plants. In some cases, nozzle cleaning could be required daily. Obviously, without this attention, the washing process would be compromised by the loss of water-flow through one or more nozzles. It is difficult for an operator to see if nozzles are working properly, so ready access to the nozzles, or use of a different, open-centred nozzle, capable of handling high levels of debris, should be considered.

In some ways, the soak tank is the heart of the cleaning process, but, on its own, the benefit of submerging a drawer for 1–2 min in contaminated water can be small. Possible changes include:

- air-jet agitation, which would also maintain aerobic conditions in the water and stress any *Campylobacter* present
- agitation from flowing water or by means of paddles
- use of a counter-current flow in the soak tank, with relatively clean water rinsing the drawers as they leave the tank
- installation of a multi-stage soak tank, which acts like a counter-current system and endeavours to allow the drawers to go from the dirtiest water to the cleanest, just before the main washing and rinsing stages.

Hot, warm or cold water?
With very few exceptions, drawers and modules are washed with cold water, which, in winter, can be below 10 ºC. In a few cases, a final rinse (after the main wash) involves warm (25–50 ºC) or hot (over 60 ºC) water. A possible hazard is

that warm water in the soak tank can encourage the growth of bacteria. Also, any application of heat can be expected to drive off remaining oxygen, thus favouring the survival of bacteria, such as *Campylobacter*, which prefer a low-oxygen environment. The real benefit of heat occurs when temperatures exceed 60 ºC. Then, there is a large reduction in the number of viable microbes, with levels diminishing even more rapidly at 70 ºC and above. Such high temperatures do bring their own problems, with large amounts of fog being generated, higher energy losses and possibly distortion of plastic drawers. A clear benefit from the use of higher temperatures is the better washing effect, especially since most detergents and disinfectants are more effective at higher temperatures. Energy considerations, based on warming a 10 kg plastic drawer by 50 ºC, imply a heat load of one MJ per drawer (specific heat capacity taken as 2000 J per kg K). This is a third of a kilowatt-hour or around 2 pence sterling in terms of heat supplied electrically (or half this amount for steam from oil or gas).

Use of chemicals at the soaking stage

The key issue is to avoid large amounts of chemicals by restricting their use to the final washing stages. This implies a multi-stage soak tank, with the bulk of the debris being removed in the first stage. The amount of cleaning agent required will relate directly to the amount of debris needing to be removed and the likelihood of it being neutralised by organic material, if too little is used.

Reduction in water carry-over and achievement of better drainage

The drawers carry dirty wash water to the next stage of the process. As mentioned previously, this can be as much as one litre per drawer and it represents a significant degree of contamination for the next stage of the process. Thus, drainage of the drawers is an important consideration and is especially the case prior to any final disinfection stage, where residual dirty water will dilute, and possibly neutralise, the disinfectant being used. Surplus water can be removed by:

- natural drainage, if sufficient time is allowed
- use of vibration to shake the drawer dry
- use of air jets to blow away excess water and impart some drying action
- drying as the last stage of the process; this is best when there is some residual heat in the drawers.

Make-up water is added directly to the system (Fig. 6.6). Although EU regulations require the water to be of potable quality, it is instantly dirtied by the main bulk of water to which it is added. If potable water is to be used, it should be directed at rinsing off dirty water from the drawers, immediately before applying disinfectant.

Disinfection processes for transport drawers

It is essential to monitor accurately the disinfectant levels used throughout the working day and to ensure that they are maintained at effective concentrations.

Fig. 6.6 Drawers leaving the soak tank to enter the main wash module (bottom corner only visible in top left of photograph). Water carried over by the drawers and the drainage water from the wash booth fall back into the main water tank via a run-down screen. The ballcock controlling the make-up water is visible near the centre of the photograph.

Problems from inadequate disinfection of drawers, modules and transport vehicles are universal (Rigby *et al*., 1980b; Carr *et al*., 1999). Experimental simulations have suggested that certain disinfectants or combinations of moderate heat and a disinfectant could be effective (El-Assaad *et al*., 1993; Ramesh *et al*., 2002, 2003), but these studies were artificial and the findings could not be substantiated in commercial operations (Carr *et al*., 1999). The most important aspect of successful disinfection is to apply a suitable concentration of disinfectant for long enough to achieve the desired result (Assanta and Roy, 2001). When organic matter is present, particularly high concentrations are needed (Ruano *et al*., 2001), and this requirement increases still further, if there are large populations of microbes in the water being used to dilute the disinfectant (Davison *et al*., 1996). All these constraints apply to the washing of transport drawers, as carried out at present. Increasing disinfectant concentrations often becomes problematical, because of corrosiveness, noxious vapours and difficulties of disposal.

In a Food Standards Agency project (Humphrey and Allen, 2002), it was found that ten of fifteen poultry companies with soak tanks used either quaternary ammonium compounds (QAC) or caustic detergents, and two companies without such tanks incorporated a caustic detergent or peroxygen disinfectant in the wash water. Thirteen companies had disinfectant spray bars

for the application of disinfectant to washed drawers, but these were not always used. Disinfectants that were used were QAC at relatively low concentrations or peroxygens at varying concentrations. These regimes are unlikely to provide effective disinfection and would need to be upgraded. Disinfection of drawers is made even more difficult by the fact that the most effective disinfectants, such as phenols or formaldehyde, cannot be used in a food processing situation.

Various methods are used for the final application of disinfectant to the cleaned drawer. However this is often ineffective as a result of:

- poor washing of the drawer, leaving a high organic load in the form of specks of debris and biofilm
- poor drainage of the drawer, leaving large amounts of entrained water that effectively dilute the disinfectant
- inadequate volume or concentration of the disinfectant being used
- inappropriate disinfectant used
- insufficient application of the spray, resulting in limited coverage of the drawer surface
- poor location of the spray, resulting in intermittent application.

Improved design and operation of module washer

Many of the potential improvements in the module washer follow from those already set out for the drawer-washing system. In particular, these include:

- use of hot water
- improved quality of re-cycled water
- use of a separate rinse cycle, using clean water
- drainage and drying options
- better disinfectant application.

There are also other issues relating to the design and deployment of modules that are discussed in the next section, since they would represent a substantial change in the current system. With modules, a key difference is that, unlike drawers, they are mostly empty space! Water jets used on drawers are easily targeted at the sides and bases, whereas, in the case of modules, a large amount of the water will miss the equipment.

6.3.4 Best practice for existing systems

Certainly, there are drawer-washing systems that are not being operated in accordance with the manufacturer's guidelines. Apart from poor maintenance of the equipment, common faults are as follows:

- infrequent checking and cleaning of spray nozzles
- infrequent removal of debris from the run-down screens
- inadequate control of the disinfection system
- insufficient or no disinfectant application
- inadequate cleaning of equipment

- over-filling of tanks
- excessive build-up of debris in the wash water
- unwarranted modifications, e.g. removal of the soak tank.

The existing plant should be run in accordance with the manufacturer's expectations. In addition to this, 'good housekeeping' should be encouraged, with the operational area and equipment being kept clean and spillages minimised. Beyond such self-evident advice, other measures that would be expected to produce improvements include the following:

- setting a minimum soaking time
- specifying a minimum amount of water to be used per drawer
- using detergent and at the correct rate
- specifying the number of drawers to be washed before the water is changed
- setting a minimum drainage time, especially after the main washing stage
- rinsing drawers with clean water, although, in this case, the demand for potable water may be prohibitive
- setting criteria for the disinfection process as either the rate of addition for the disinfectant used or the microbial count level to be achieved.

6.4 Pre-slaughter lairage systems

6.4.1 Reducing stress

Following transportation, lairage facilities should allow the birds to be rested before slaughter. Controlled ventilation systems, as described in Section 6.3, are equally applicable here for bird-welfare reasons and may have a worthwhile effect on faecal contamination, so that the birds remain cleaner or, at least, do not become appreciably dirtier. Also, the faecal matter may be removed more readily by the washing system. At present, however, these possibilities are purely speculative.

6.4.2 Improved biosecurity and cleanliness

Many lairage systems do not separate properly the dirty areas from the clean ones. Dirty lorries arrive fully-loaded from farms, to be unloaded in the same area and with the same forklift trucks as those used to load the freshly-cleaned transport modules onto clean lorries. In future planning of processing plants, consideration should be given to separating completely the dirty, unloading operation in the lairage from the clean area, where the transport modules and lorries have been cleaned and disinfected. It is also important to restrict the flow of effluent, faeces and other debris to the dirty side of the process, so that it does not contaminate the clean side. In addition, it is obvious that personnel should be prevented from going directly from the lairage to other parts of the processing plant. Finally, the whole lairage area should be kept as clean as possible and, especially, dry.

6.4.3 Problems with airborne particles

As discussed earlier, *Campylobacter* has been found in air streams and transmission of *Salmonella* has been linked to air flow under controlled conditions. The lairage can contain a high density of birds and may, under appropriate conditions of wind or ventilation, provide the source of a plume of contaminated particles that could reach cleaned equipment or even sensitive areas of the processing plant, such as the packing lines, where finished carcasses are exposed to the air.

6.5 Future trends

6.5.1 On-farm slaughter: a discussion point

One aspect that has not yet been considered in this chapter is the requirements of small production systems. In the UK, free-range poultry, whether or not reared organically, are widely present. Because of their uncontrolled environment, the birds are particularly prone to infection with *Campylobacter*, which they then carry in large numbers. A point for discussion is whether these birds should be killed and (partly) processed at the farm of origin. This would have bird-welfare benefits and the reduced stress and minimal time between catching and slaughter may allow contamination of carcasses with pathogens to be reduced. Lower levels of stress may minimise shedding of pathogens, while reduced handling of the birds and avoidance of holding them in confined transport containers may significantly reduce overall faecal contamination. This approach is unlikely to be suitable for large flocks from intensive systems, where other issues to do with chilling, water supply and waste disposal would become more prominent.

6.5.2 Biosecurity

Knowledge of biosecurity at the farm and the processing plant is widely acknowledged to be important. There is, however, a problem in ensuring that all staff follow all the precautions all of the time. This can be facilitated by using systems that provide built-in biosecurity. The broiler harvester is such a system, as discussed earlier. The one disadvantage is that it is a difficult machine to clean and disinfect satisfactorily and, in future, more attention must be given to simplifying the design for ease of cleaning. Also, unloading the birds from lairage and re-loading clean crates will require some careful design to avoid cross-contamination from effluent, contaminated airborne particles and from dirty lorries and forklift trucks.

6.5.3 Improved treatment of recycled water in the washing process

A major weakness in the design of current washers is the inadequate treatment of water that is recycled around the process. A build-up of suspended matter is inevitable, along with an increase in microbial numbers. The run-down screens

used are cheap and simple to operate, but they can only remove coarse particles (over 1 mm in diameter). If kept clean, they remain a useful first-step in treatment, but further stages should be considered to improve water quality. This implies taking water out of the system and passing it through a dedicated treatment-loop that meets a specific objective.

Sedimentation
This is a simple concept, requiring a settling tank with minimal disturbance. Flocculants may be mixed in before the wash water is fed into the vessel. Separation by natural gravity leads to the production of a sludge concentrate at the bottom of the vessel and a clarified supernatant at the top. If the sludge is not well concentrated, it can represent an excessive proportion of the original volume and further concentration (e.g. by use of a centrifuge or hydrocyclone) may be necessary. The supernatant should contain only a low level of suspended matter, making the option of subsequent filtration by plate and frame filter a realistic choice.

Membrane process
The clarification process can only affect the concentration of microbes if filters of very small pore-size are used, such as ultrafiltration membranes. This requires the wash water to have been clarified before filtration, because such membranes are susceptible to blockage. It is doubtful that the benefit of such an elaborate treatment justifies the cost of recycling wash water for poultry drawers.

Thermal treatment
Although recognised as effective, the technology is often overlooked, owing to the perceived high cost. However, this is not necessarily the case, if heat recovery is used to bring running costs down to below 30 pence sterling per tonne of water. Pre-clarification may be included for removal of particulate matter, but is not critical for thermal treatment, which returns essentially sterile water to the system for further washing purposes.

6.5.4 Use of sonication at the soaking stage of the cleaning process

The expected benefit from sonication is in loosening attached debris (including microbes), which suggests that it may be particularly useful in the soak-tank. Limitations of the technology include the tendency for plastics to absorb the energy (and thus negate its effect) and the need for the water medium to be free of dissolved air for good transmission.

6.5.5 Use of brushes in drawer cleaning

The use of brushes to remove dirt from transport drawers is limited, because of the many surfaces to which it is difficult to gain access. The attraction lies with the possible removal of biofilm layers and, where surfaces are readily accessible,

this seems a reasonable expectation. Brushes used in a submerged location, such as the soak tank, might also be expected to achieve further benefit by causing agitation. The simplest system would be a single rotary brush to clean only the base of the drawer.

6.5.6 Steam drying and disinfection options in drawer cleaning

The use of steam in food production always appears to be a relatively costly process; also, its use in the present context would require containment of the fogs produced. However, it does allow chemical-free disinfection and, if used early on in the drawer-cleaning process, may provide a thorough loosening of attached faecal matter. Treatment of surfaces with steam is potentially more efficient than that involving chemical sprays, in that all surfaces are in contact with the vapour. It will be important that the temperature of the treated surface reaches 70 ºC or more and is held at this level for at least one minute to ensure a large reduction in microbial contamination. This implies that the drawer should be in the steam tunnel for more than this period of time, perhaps for the same period as that used for soaking. An alternative approach might be to steam-treat the drawers when they have been returned to the module.

The poor conductivity of the plastic material should minimise steam consumption. In fact, only the surface would be heated, perhaps equating to no more than 20% of the drawer volume. Assuming this to be the case, there would be a running cost of less than 0.5 pence sterling per drawer, depending on the efficiency of the treatment. Larger amounts of steam could allow some drying of the drawers on the basis that drainage is enhanced and there is subsequent evaporation of moisture on entering a cooler, dryer environment outside the steam tunnel. The extent of this benefit needs to be evaluated, as does the potential shortening of the useful life of the drawer, as a result of repeated exposure to thermal cycles in the cleaning process.

6.5.7 Use of ultraviolet radiation in the disinfection of drawers

The use of ultraviolet (UV) light to kill microbes is already well-established in other industries, such as that involving treatment of water for drinking purposes. Treatment of the drawer surface in an appropriate manner might be expected to have a similar effect on contaminating microbes. The method does have the attraction of easy deployment, with little more than the fitting of a row of lamps around the final stages of drawer washing, and some shielding for operator protection. However, there are possible drawbacks to such an approach that point to the need for further research:

- UV will only penetrate transparent surfaces; it will have no effect on microbes that are ‘protected’ by any debris remaining after cleaning (it is essential that the drawer is visibly clean and well-drained prior to ultraviolet treatment)
- the treatment would need to be maintained for a significant period of time, implying a series of lamps, possibly in a treatment tunnel

- any surface that is in a shadow will be untreated; for transport drawers, this may mean a high proportion of all surfaces, unless many lamps are used to illuminate from various directions
- UV will shorten the life of the plastic from which the drawer is made.

6.5.8 The concept of a drying room for washed drawers

In dry conditions, bacteria cannot multiply and, indeed, numbers can be expected to fall as many die off. Hence, drying the drawers prior to returning them to the lorry is an effective way of reducing microbial levels (including those of *Campylobacter*). The effect can be improved by extending the period during which the drawers are dry; however, this would require additional storage space and drawers. The cost of this approach would be for the additional space required and, more importantly, the extra drawers required. At around £50 sterling per drawer and five per minute passing through the system, a drying room holding drawers for four hours would require drawers costing a total of £60,000 sterling.

6.5.9 Modules: design and deployment issues

For the transport drawers, soiling of the surface remains an uncontrollable part of the process, but this is not the case for modules, since some control may be possible at the farm, depending on how they are used. For example, the problem of debris compacting on the base may be reduced if the module is fitted onto a 'shoe' on arrival at the farm. Once the module is full, the shoe could be removed and retained at the farm. The effect of mechanical catchers on deployment of the modules is discussed in Section 6.2.2.

6.6 Sources of further information and advice

Websites

The Internet now provides a wide source of information and allows lines of information to be followed. In particular, searches on poultry biosecurity will give hints on what to consider.

Trade and professional associations

Trade and professional associations have lists of consultants and other information that is primarily intended for the use of their members. Increasingly, the topic may be seen as an environmental issue, and organisations such as the Society for the Environment, which is an umbrella organisation encompassing professional bodies, including the Chartered Institution of Water and Environmental Management (CIWEM), Institute of Environmental Management and Assessment (IEMA) and Institution of Agricultural Engineers (IAgrE), may be able to suggest individuals with appropriate experience.

Research and development organisations

There is no R&D organisation in the UK that deals specifically with this topic, but there are a few that have departments as off-shoots of their main activities and carry out research that may be relevant. Of possible interest in this respect is the Campden and Chorleywood Food Research Association, and the Health and Safety Laboratories. The former has staff dealing with hygiene in food factories, and the latter undertakes research, particularly in relation to operator health. The company known as ADAS and the Veterinary Laboratories Agency have knowledge of farm biosecurity and disinfection procedures respectively. Although being closed in 2006, the Silsoe Research Institute has staff that have been involved in all of the aspects discussed here, and they anticipate continuing to use their expertise.

Universities and colleges

Much research is carried out in UK universities, especially at PhD level, and a few have specialist departments that relates to the subject area, e.g. University of Bristol and the Scottish Agricultural College, Auchincruive.

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7

Ensuring the safety of poultry feed

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7.1 Introduction

The nutrient composition of feed ingredients and the formulations used commercially are important for bird growth, nutrition and egg production, but are only part of the poultry-feed matrix. From the microbiological standpoint, feeds can serve as transmission vectors for a variety of benign and harmful organisms. This is because such feeds can contain a fairly diverse range of microbes that originate from contact with, and exposure to, multiple sources in the environment, including aerosols, soil, water and biological vectors. The organisms of concern are a risk to poultry and/or humans and include viral, bacterial and fungal contaminants. Harm from these organisms can be mediated by direct contact with the organism in question and subsequent expression of some form of pathogenesis in the avian host. Damage to the host can also result from exposure to a microbial toxin that remains in the feed, whether or not the organism responsible is still present. In addition, feed or feed ingredients can be the origin of foodborne pathogens that colonize the intestinal tract of the bird, following consumption. This can lead to an asymptomatic carrier state in the avian host and a potential public health hazard from the ingestion of contaminated poultry meat or eggs. This chapter focuses on the mycotoxin-producing fungi and bacterial foodborne pathogens that can be associated with poultry feed. The following sections include a discussion of the incidence of these organisms in poultry feeds, factors that influence the levels present and potential control measures.

7.2 Incidence of toxigenic fungi and foodborne bacterial pathogens

7.2.1 Fungi in poultry feeds

Cereal-grain fungi that produce mycotoxins are typically separated into two distinct groups, namely those that become associated with grain prior to harvest and are referred to as 'field fungi' and others that are acquired during storage of grain and are classified as 'storage fungi' (Miller, 1995). Regardless of their origin, the toxigenic fungi represent a potential threat to feed quality and can cause musty or sour odors, losses in dry matter and nutrients, caking, mycotoxin formation and, ultimately, a reduced monetary value (Beuchat, 1978; Sauer *et al*., 1992; Barney *et al*., 1995). A considerable amount of research has been carried out on fungal populations on grain, methods for their detection and enumeration in the field and production of mycotoxins, which can have an adverse effect on growth performance in poultry.

Mycotoxins belong to a broad class of secondary fungal metabolites that are produced by *Aspergillus*, *Penicillium* and *Fusarium* spp. (Hesseltine *et al*., 1976; Wilson and Abramson, 1992; Dänicke, 2002). The mycotoxins of toxicological and agricultural significance include aflatoxins, ochratoxins, trichothecens, zearlenone and fumonisins (Miller, 1995; Dänicke, 2002). However, the frequency of particular mycotoxins on cereal grains varies, because the fungi present can differ in the toxins they produce, although some produce the same toxin (Dänicke, 2002). Consequently, the bird can be exposed to several mycotoxins simultaneously at differing concentrations, resulting in complex effects, including additive and synergistic responses (Dänicke, 2002). Mycotoxin production and occurrence in poultry feeds have been studied extensively, as well as the consequences for avian health and well-being. When considering poultry feed, the toxicological aspect is further complicated by the fact that both feed grains and other ingredients may contain mycotoxins (Burditt *et al*., 1983; Mahmoud, 1993). With individual cereal crops, such as corn, the incidence of different fungi can be influenced by a myriad of factors, including environmental storage conditions, moisture level in the harvested grain and exposure to insect pests (Barney *et al*., 1995). Poultry consuming mycotoxins may exhibit a number of physiological responses as a result of the direct pharmacological effects on specific organs, such as the liver, kidney or heart, or the immune system (Dänicke, 2002). When mycotoxins are deposited in the tissues of the bird, there is a public health risk from either the mycotoxins themselves or their biotransformation products *in situ* (Bullerman, 2001; Dänicke, 2002). In addition to direct toxicological effects, fungal growth on feed ingredients and in feed mixes can modify the nutrient composition of feed and subsequently reduce nutritional quality, leading to intermittent changes in bird performance (Vieira, 2003).

Decontaminating poultry feed that contains mycotoxin remains a difficult process, with few viable options. Although heating, irradiation and ultraviolet treatment, as well as the use of chemical compounds, such as chlorine, hydrogen

peroxide, alkali and ozone, have potential, these approaches appear to have limited applicability on a large scale. Therefore, research interest is now directed more towards biological methods of detoxification (Dänicke, 2002; Vieira, 2003). Reducing the overall level of mycotoxin in a feed ingredient by mixing with uncontaminated material represents an economical and readily-available management tool to avoid potentially toxic levels, but is considered a complicated process under commercial conditions (Vieira, 2003). Development of additives that would detoxify mycotoxin-contaminated feed during consumption and subsequent passage through the avian intestinal tract could avoid any risk to the bird. The concept of adding binding agents to mycotoxin-contaminated feed, as a means of sequestering the toxin and subsequently reducing its bio-availability in the gastro-intestinal tract, has been examined extensively as a practical solution to the problem (Huebner *et al*., 2004). Both non-biological and biological compounds have been screened *in vitro* and/or *in vivo* for potential binding capability and specificity. Substances such as hydrated calcium alumino-silicates, zeolites, natural clays, bentonites, activated charcoal and *Saccharomyces cerevisiae* cell-wall polysaccharide have been shown to bind certain mycotoxins *in vitro* and, in some cases, improve bird performance (Phillips *et al*., 1988; Kubena *et al*., 1990a,b; Huwig *et al*., 2001; Vieira, 2003). However, not all absorbents are equally effective in binding different mycotoxins *in vitro* and this may account for the varying levels of protection *in vivo* (Kubena *et al*., 1990a; Huff *et al*., 1992; Ramos *et al*., 1996; Dänicke, 2002; Vieira, 2003).

Since mycotoxins are considered to be relatively stable compounds and therefore capable of persisting in feeds for extended periods of time, preventing fungal growth, and hence toxin production, would appear to be the best approach for control purposes (Vieira, 2003). The goal would be to implement management practices that ensure unfavorable conditions for the growth of fungi, without sacrificing the nutritional quality of the feed. From the standpoint of field fungi, low initial moisture in corn seed, adequate pest management and high temperature have all been shown to limit growth of some mycotoxin-producing fungi (Barney *et al*., 1995). Using plant breeding techniques to create cereal-grain varieties that are resistant to mycotoxin-producing fungi may have potential, but will require more in-depth understanding of the biological association between each fungus and the plant hybrid (Miller, 1995). Chemical compounds that inhibit fungal growth have been employed traditionally to preserve grains for animal use, and include organic acids, sorbic acid, ammonia, sodium chloride, sodium propionate and ammonium iso-butyrate (Vieira, 2003). Several organic acids, including formic, acetic, propionic and butyric acids, are effective for controlling fungal growth on a variety of cereal grains, with propionic acid being the most widely used (Herting and Drury, 1974; Vieira, 2003). However, Vieira (2003) has pointed out that the need to use organic acids for stored grain depends, to some extent, on whether the water activity of the grain is high enough to support fungal growth in the first place.

7.2.2 Bacteria in feeds

Unlike fungi, where the main threat to the bird from consumption of contaminated feed is due to mycotoxin production, bacterial pathogens themselves are the main hazard. However, such pathogens do not always produce disease symptoms in the bird. On the other hand, when bacteria that are pathogenic to humans become associated with poultry feed, their potential as an inoculum for birds consuming the feed is considerable. Subsequent colonization of the avian gastro-intestinal tract can lead to contamination of meat and eggs and, consequently, is a food safety issue. Contamination of poultry feed and ingredients by foodborne pathogens can come from a multitude of sources, including air, water, animal/wildlife vectors, fecal material and soil. The source of the contamination may influence the types of bacteria present and their potential for survival. For example, fecal material will often contain pathogenic bacteria that have become adapted to the intestinal ecosystem. This is characterized by the presence of sufficient moisture and nutrients for microbial growth, but, on the other hand, it can also be hostile, combining a low oxidation-reduction potential with other factors in a highly selective, competitive environment that can limit the persistence of some organisms. When mixed with poultry feed, the organisms in fecal material meet a new set of environmental challenges until they are able to colonize another host. In the following sections, consideration is given to foodborne bacterial pathogens that are known to have some association with the intestinal tract of poultry and are potentially present in poultry feed.

Clostridium perfringens

Cl. perfringens is a common organism in the intestinal tract of poultry and has been linked to sporadic incidents of necrotic enteritis (Truscott and Al-Sheikhly, 1977; Cowen *et al*., 1987; Tschirdewahn *et al*., 1991). An array of other poultry diseases has also been associated with *Cl. perfringens*, including gas edema, gizzard erosion and gangrenous dermatitis (Gerdon, 1973; Hofacre *et al*., 1986; Fossum *et al*., 1988; Craven, 2000). Gangrenous dermatitis, involving a severe form of enteritis, may result in a mortality rate of 1 to 2% per week in turkeys (Carr *et al*., 1996). In addition, *Cl. perfringens* is an opportunistic colonizer of the intestine and Truscott and Al-Sheikhly (1977) noted that feeding high levels of fishmeal (*c.* 50%) resulted in persistent enteritis in poultry from this organism. Dietary stress and changes in diet may also be a factor. Craven (2000) compared rye- and corn-based diets in a *Cl. perfringens* challenge study on broiler chicks and observed that the rye-based diet increased populations of *Cl. perfringens* to levels comparable with those of chickens with necrotic enteritis.

Being able to form endospores enables the organism to resist adverse environmental conditions, such as low pH, high temperature and low water activity, that are unlikely to support vegetative growth in feed under normal storage conditions. *Cl. perfringens* can be isolated from pelleted feed, because the spores are highly resistant to heat, although re-contamination after pelletting from sources such as soil cannot be excluded (Greenham *et al*., 1987). However, there is known to be variability in heat resistance that can be influenced by both

genetic and environmental factors (McClane, 2001). Controlling *Cl. perfringens* in feed appears to require strategies similar to those used in food processing and would focus on keeping the organism in the vegetative state, and in low numbers, by preventing spore germination and outgrowth during storage of the feed. Nevertheless, vegetative cells of *Cl. perfringens* are also somewhat heat-tolerant, particularly food-poisoning strains. These were found to be twice as heat-resistant at 55 ºC as other strains (Sarker *et al*., 2000; McClane, 2001). What impact this might have on the effectiveness of the pelletting processes used in feedmills remains to be determined.

Salmonella

Feed continues to be regarded as an important source of *Salmonella* infection for poultry and other food-animal species. Thus, *Salmonella* has been isolated from feeds for more than 60 years in a wide range of geographical locations (Hacking *et al*., 1978; Williams, 1981a; Jones *et al*., 1991b; McChesney *et al*., 1995; Veldman *et al*., 1995; Davies and Wray, 1996; 1997; Shirota *et al*., 2001; Crump *et al*., 2002; Alvarez *et al*., 2003; Whyte *et al*., 2003; Jones and Richardson, 2004). In addition, the continued trend for larger numbers of birds to be housed in confinement increases the likelihood that more birds will be infected almost simultaneously. This risk may be amplified further by increased feed-mixing activity and incorporation of a wider range of ingredients from various sources, as well as the expansion of global trade in animal feed and feed-ingredient commodities (Crump *et al*., 2002). Of the individual feed ingredients, animal proteins are more likely than others to be contaminated with *Salmonella* (McChesney *et al*., 1995; Crump *et al*., 2002). The range of serotypes isolated from poultry that are likely to be associated with feed as a point of origin has been difficult to determine, due the limited number of surveys carried out in conjunction with commercial feed operations, and the inability to serotype all the isolates obtained (Crump *et al*., 2002).

The continued prevalence of *Salmonella*-contaminated feed can be partly attributed to the distribution and dissemination of the organisms, with a wide range of biological vectors being involved (Klowden and Greenberg, 1976; 1977; Henzler and Opitz 1992; Kopanic *et al*., 1994). Once inoculated into dry feed, *Salmonella* can survive for periods of several months to more than a year (Williams and Benson, 1978; Juven *et al*., 1984; Davies and Wray, 1996; Ha *et al*., 1998a). Detection of low numbers in feeds still represents a risk because, for some *Salmonella* strains, less than one organism per gram of feed is sufficient to colonize chicks up to seven days of age (Schleifer *et al*., 1984).

7.2.4 Other pathogens

Other pathogens may also be present in finished feeds and grains, although their transmission routes are less clear. Thermophilic *Campylobacter* spp. are regarded as important foodborne pathogens that are often associated with poultry, but any vertical transmission tends to be obscured by rapid horizontal spread through an

infected flock (Shanker *et al*., 1990; Humphrey *et al*., 1993; Keener *et al*., 2004). Poultry feeds have been surveyed for thermophilic campylobacters (Genigeorgis *et al*., 1986; Jones *et al*., 1991a; Whyte *et al*., 2003; Saleha, 2004) and the infrequent and/or undetectable levels reported are believed to be due to the sensitivity of these organisms to the desiccated nature of most manufactured poultry feeds (Blaser *et al*., 1980; Luechtefeld *et al*., 1981; Doyle and Roman, 1982; Whyte *et al*., 2003). Potential routes of infection for poultry appear to include direct contact with contaminated feces or drinking water (Hänninen *et al*., 1988; Humphrey *et al*., 1993; Pearson *et al*., 1993). The primary route, however, remains to be elucidated and this may be complicated by the possible presence of a viable, but non-culturable, coccoid form of *Campylobacter* that could represent a survivor state capable of colonizing chicks (Rollins and Colwell, 1986; Ziprin, 2004). If such a physiological form is part of the normal life-cycle of *Campylobacter*, then poultry feed could be one of the transmission routes.

Listeria spp. have been found in poultry feeds both before and after heat processing (Blank *et al*., 1996; Whyte *et al*., 2003). It remains unclear how much of a factor *Listeria*-contaminated poultry feed is in the overall transmission of foodborne *Listeria*. Whyte *et al*. (2003) noted that, after heat treatment, much of the environment, including dust from milling equipment and the air inlet of the pellet cooler, was highly contaminated with *Listeria* spp., suggesting that re-contamination of processed feed from the mill environment could easily occur.

7.3 Decontamination treatments for feed

7.3.1 General concepts and principles

Feed used in poultry production comprises a combination of nutritionally important components which, depending on the degree of physical and chemical processing, provide a certain level of nutrients for the broiler or laying hen (McNab and Boorman, 2002). As well as the need to maintain nutritional quality, the degree of processing used depends upon several factors, including the required storage time, economic considerations, food safety requirements and other more specific or situational demands (Dean, 2002; McCracken, 2002). Emphasis on a particular set of criteria depends upon the type of feed, the ingredients involved and any commercial or regulatory requirements deemed necessary to ensure nutritional quality and freedom from agents of public health concern (Dean, 2002). Maintaining a high nutritional quality and low public health risk requires a balance between optimum nutrient availability and a minimal risk from pathogenic microorganisms in the feed (Cox *et al*. 1986; McCracken, 2002). To achieve this balance, numerous chemical additives and physical treatments have been examined and, in some cases, implemented. Approaches used to limit exposure of poultry to pathogens in feed involve either treatments aimed at reducing feed contamination directly, or indirect methods, using the feed as a carrier for either chemicals or biological products that will target pathogens once they reach the gastro-intestinal tract.

The most common approach is to reduce the initial bacterial load in the feed itself by some chemical or physical means. This can be accomplished by adding chemical compounds, such as acids or other substances that possess known antimicrobial properties. Pathogen reduction can also be achieved by physical processes, such as heating or by removal of water during drying. Regardless of the approach used, alteration of the feed matrix to achieve a certain level of pathogen reduction can result in a feed environment that is less than optimal for supporting bacterial growth. Therefore, growth of any remaining pathogens in the feedstuff, or those acquired by subsequent re-contamination, should be minimal, if wetting of the feed occurs. However, most organisms have evolved mechanisms that respond to sub-optimal growth conditions and therefore post-processing survivors or re-contamination of feed may continue to be problematic. The following sections consider some of the more common feed treatments that have been studied.

7.3.2 Thermal processes

Of the available processes for destroying *Salmonella* in feed, heat treatment during the terminal stages of processing has always been considered the simplest and most economical method (Wilder, 1969, Williams, 1981c). Several studies have verified the effectiveness of the heat treatment used in pelletting for reducing *Salmonella* contamination (Mossel *et al*., 1967; Stott *et al*, 1975; Vanderwal, 1979; Cox *et al*., 1986; McCapes *et al*., 1989; Jones *et al*., 1991b; Veldman *et al*., 1995). The last-mentioned concluded that, for heat to be effective in the pelletting process, the temperature must exceed 80 ºC, so that *Salmonella* populations are reduced to undetectable levels. However, most of the feedmills they surveyed in The Netherlands were using temperatures within the range 70–75 ºC. In a study of *Salmonella* contamination at US feedmills, and comparing days of sample collection and seasonal changes, Jones and Richardson (2004) concluded that feeds should be heated to 85 ºC to ensure freedom from *Salmonella*. Several factors influence the effectiveness of pelletting, including certain processing variables, such as moisture level in the feed and treatment time and temperature, as well as nutritional concerns about heat damage and loss of essential nutrients (Williams, 1981c; Veldman *et al*., 1995; Himathongkham *et al*., 1996). It is possible that higher temperatures could be achieved with extrusion processes and these might be more effective at eliminating *Salmonella* (Jones and Richardson, 1996).

Bacterial responses to heat are also important. In general, bacteria tend to be more heat-resistant as water activity decreases, and this was demonstrated for *Salmonella* Senftenberg in meat and bone meal (Liu *et al*., 1969; Farkas, 2001). Varying levels of thermo-tolerance occur among *Salmonella* serotypes and the expression of heat-resistance varies during the different phases of growth (Ng *et al*., 1969). More recently, it has been shown that exposure of *Salmonella* Typhimurium to sub-lethal temperatures can increase heat resistance through the formation of heat-shock proteins (Mackey and Derrick, 1987; Farkas, 2001).

The physical form of the feed pellet may also be an indirect factor, since Mikkelsen *et al*. (2004) found that feeding pigs a coarsely-ground, non-pelleted meal increased levels of undissociated lactic acid in the gut to those causing reduced survival of *Salmonella* Typhimurium. Whether there is a similar relationship between the physical form of the feed and stimulation of fermentation responses in the avian gastro-intestinal tract that would be antagonistic to *Salmonella*, remains to be determined.

Despite the efficacy of heat treatment there is always the risk of re-contamination during milling, transport and storage of feed (Hinton and Linton, 1988). Whyte *et al*. (2003) obtained comparable *Salmonella* isolation rates from samples of feed and environmental dust that were collected from both pre- and post-treatment areas of a commercial feedmill, and even higher levels from feed transport vehicles. Given the large number of potential environmental sources and vectors of *Salmonella* that may come into contact with feed, this is not too surprising. Jones and Richardson (2004) noted that the accumulation of dust containing *Salmonella* near the pellet mill could essentially negate any beneficial effect of the pelletting process. This can be a persistent problem, if the design of the mill does not allow adequate separation of the pre- and post-treatment areas and includes, for example, a shared ventilation system (Jones and Ricke, 1994; Jones and Richardson, 1996; Whyte *et al*., 2003). Likewise, preventing raw ingredients from coming into direct contact with heat-treated feeds would be considered critical (Jones and Ricke, 1994; Jones and Richardson, 1996).

7.3.3 Chemical disinfection

Overview

In view of the risk of re-contamination after heat treatment (Hinton and Linton, 1988), there has been considerable interest in developing chemical disinfectants that would both reduce existing levels of pathogens in poultry feed and minimize re-contamination. Over many years, numerous compounds have been considered for these purposes, including acetic acid, buffered propionate, citric acid, ethyl alcohol, formaldehyde, formic acid, isopropyl alcohol, lactic acid, phosphoric acid, propionic acid, zinc acetate and zinc propionate (Khan and Katamay, 1969; Duncan and Adams, 1972; Vanderwal, 1979, Williams, 1981c; Hinton and Linton, 1988; Humphrey and Lanning, 1988; Rouse *et al*., 1988; Ha *et al*., 1997; van Immerseel *et al*., 2002; Park *et al.*, 2004). Although most of these compounds were selected originally because of their effectiveness against particular feed contaminants, such as fungi, they must still be tested under typical field conditions.

Based on studies with both fungi and bacteria, it is apparent that several criteria need to be satisfied for optimum use of a chemical disinfectant to treat feed routinely in the field. Candidate feed disinfectants must effectively reduce pathogen contamination in the presence of large concentrations of organic matter and competing microbes. Ideally, this will have been demonstrated in an

initial screening trial but, if not, the candidate compound should be added to a sample of feed that has been inoculated with the target pathogen to provide a representative feed microcosm. Such microcosms should be as similar as possible to field conditions. For example, if there is concern that a certain ingredient, such as a particular type of protein (cereal grain or an animal by-product), may counteract the antimicrobial properties of the disinfectant, then the test microcosm should contain the suspect protein. Also, the pathogen response should be examined under different environmental conditions by varying, for instance, water activity and storage time and temperature, in the presence of a typical, indigenous microflora.

To facilitate recovery of the target pathogen from a non-sterile microcosm, a marker strain of the pathogen is often used. This will have been genetically modified to contain a highly specific pattern of antibiotic resistance or to have luminescent or fluorescent properties that allow differential enumeration against the background microflora. It is important to avoid using sterilized feed in the test, since there may be organisms present normally that could take up the candidate disinfectant or metabolize it into an ineffective form. Failure to recognize these possibilities could result in an overestimate of the effectiveness of the test compound. In relation to the pathogen inoculum, account should be taken of possible differences in response for different physiological states. For example, as discussed previously, when feed is contaminated with *Salmonella* at the mill, the organism is unlikely to be in a physiological state comparable to that occurring under ideal conditions in the laboratory. Also, because some strains of *Salmonella* are known to survive longer in feed at low water activity (Carlson and Snoeyenbos, 1970; Juven *et al*., 1984), the method of preparing the inoculum for the test must avoid any subsequent effect on the water activity of the inoculated feed. In generating *Salmonella* strains and other bacterial inoculants, methods have been devised that involve a nutritionally-inert material, such as chalk particles, as the storage matrix for the organism and as the eventual carrier for inoculating the feed matrix (Hoffmans and Fung, 1993). Such approaches allow the assessment of antimicrobial activity, using organisms that are already in a state where they are adapted to slow growth. This could be important, as the organisms may respond differently from those growing optimally.

Hinton and Linton (1988) have pointed out that, in addition to being non-toxic to humans and animals, chemical disinfectants must be stable in the feed until it is consumed and subsequently metabolized without being converted by the animal to toxic substances. Obviously, compounds that are unstable under field conditions or subject to oxidation would be unsuitable. Since any chemical would probably be applied at the point of mixing (so as to disperse it throughout the feed), the additive should not be corrosive enough to damage the equipment to an extent that would outweigh the economic benefits of pathogen reduction. Also, a disinfectant should be easily and conveniently stored, so that it cannot pose any serious threat to either the surrounding environment or farm personnel.

Organic acids

Organic acids possess many of the attributes of an ideal feed disinfectant and much of the focus in designing practical treatments for feeds has involved their use. Many occur naturally in certain human foods and have a long history of being added to foods as preservatives (Davidson, 2001). Short-chain organic acids, such as acetic, propionic and butyric, have proved to be effective as fungistats in stored grain and have been used to prevent fungal growth in feeds given to poultry and other animals (Herting and Drury, 1974; Stewart *et al*., 1977; Paster, 1979; Dixon and Hamilton, 1981; Christensen and Sauer, 1982; Lin and Chen, 1995; Ricke, 2003; Vieira, 2003). The antibacterial mechanism(s) are considered to be primarily pH-driven, since these are weak acids and lowering pH would favor the undissociated form, which can easily cross the cell membrane. Once inside the cell, the acids reduce the near neutral pH value of the cytoplasm and may have other consequences, such as membrane damage and/or anion accumulation (Cherrington, *et al*. 1991; Russell, 1992; Davidson, 2001; van Immerseel *et al*., 2002; Ricke, 2003). Once consumed by the bird, however, the acids appear to be metabolized rapidly. Hume *et al*. (1993) confirmed this by following the fate of ^{14}C labeled propionic acid in the chicken and demonstrating that most of the acid consumed was absorbed in the foregut (crop, gizzard and proventriculus), without reaching the lower intestinal tract.

When organic acids are added to poultry feed to eliminate either naturally occurring *Salmonella* or inoculated 'marker' strains, the results have been inconsistent and, in some cases, the acids have had relatively small antimicrobial effects (Khan and Katamay, 1969; Duncan and Adams, 1972; Vanderwal, 1979; Hinton and Linton, 1988, Humphrey and Lanning, 1988; Rouse *et al*., 1988; Ha *et al*., 1998b). However, incorporation of an acid in the feed can still reduce *Salmonella* infection in the chicken (Hinton and Linton, 1988; Thompson and Hinton, 1997). These studies suggest that there could be some variation in the timing and location of the antimicrobial effect, whether during feed processing or following consumption of the processed feed by the bird. The ability of organic acids to control microorganisms in poultry feed is dependent on several factors that may influence the effective concentration to which bacteria or fungi are exposed in the feed matrix. Propionic acid, for example, can display inconsistent antifungal properties, due possibly to buffering effects and subsequent conversion to a less active form by reaction with protein ingredients, such as soybean meal, as well as batch-to-batch differences in feed composition (Dixon and Hamilton, 1981; Tabib *et al*., 1984). It has been suggested that acids are largely inactive in dry feed and are activated only when the feed is consumed by the bird and becomes hydrated by mixing with saliva (Duncan and Adams, 1972; Hinton and Linton, 1988). Moreover, it has been suggested that, in dry feeds, there is limited contact between a contaminating organism and the chemical disinfectant present (Duncan and Adams, 1972).

There are several options for enhancing disinfection of feed with organic acids. One approach would be simply to increase the amount of acid, but this may present a different set of problems. Increasing the concentration of

propionic acid may reduce feed palatability and intake as well as increase the requirement for vitamin B_{12} (Ryś and Koreleski, 1974; Cave, 1982, 1984). There is also the possibility that raising the concentration of a short-chain fatty acid, particularly at neutral pH, may induce acid resistance in *Salmonella* (Ricke, 2003). A longer exposure time to the acid is possible, but this may not be practicable in commercial circumstances (Leeson and Marcotte, 1993). Potentially synergistic combinations of acids, such as formic and propionic together, have been shown to reduce *Salmonella* shedding in hens and broiler chickens (Hinton and Linton, 1988; Thompson and Hinton, 1997). A more effective strategy might be to incorporate two acids with different types of antimicrobial activity. Matlho *et al*. (1997) increased the destruction of *Salmonella* Enteritidis in poultry feed by combining heating with the incorporation of propionic acid. Although organic acids added directly to feed appear to be active only in the upper parts of the avian digestive tract, they would be more effective if the antimicrobial activity persisted throughout the tract (Dibner and Buttin, 2002). Significant reductions in *Salmonella* colonization have been achieved in a number of studies, using either carriers coated with mixtures of propionic and formic acids (Hinton and Linton, 1988; Iba and Berchieri, 1995; Berchieri and Barrow, 1996) or micro-encapsulation of butyric acid (van Immerseel *et al*., 2004).

7.4 Safe management of feed production

Although there are several options for controlling pathogens in feeds, it is still necessary to identify those sites at the mill that are most likely to be contaminated and others that would be considered less of a risk. However, given the complexity of feed manufacturing and processing, any attempt to isolate (let alone quantify) pathogens from feeds in a representative manner becomes a difficult logistic exercise. A more effective approach is to identify the key sites where contamination is most likely to occur. Currently, Hazard Analysis Critical Control Point (HACCP) plans are required for some parts of the US poultry industry, such as processing plants (de Graft-Hanson, 2003). For certain pathogens in feeds, such as *Salmonella*, the use of HACCP programs is being encouraged, with the goal of eliminating *Salmonella* from feedstuffs (Crump *et al*., 2002). In countries such as Sweden, HACCP programs for animal feeds have been implemented and, once incorporated into the overall surveillance and control system, have proved to be highly successful in eradicating *Salmonella* (Crump *et al*., 2002; Hopp *et al*., 1999). HACCP programs for US feedmills were proposed more than ten years ago, but only a limited number of studies have documented thoroughly the contamination of feed during manufacture (Jones and Ricke, 1994; Jones and Richardson, 2004).

Implementation of the HACCP system in US feedmills is not necessarily a small undertaking, because several issues relating to sampling and pathogen detection would make it difficult to generate the large data sets needed to

pinpoint Critical Control Points (CCPs) in feedmills. This has been the reason for the few US studies that have been carried out in the past ten years and their limited nature. In particular, the quantity of feed needing to be sampled presents a practical problem. In commercial feedmilling operations, several tons of feed may be produced every hour (Jones and Ricke, 1994). The sampling problem is highlighted by the fact that only one *Salmonella* cell per gram of feed is required to colonize young chicks and only low levels of *Salmonella* contamination are likely to be present in the large amounts of feed being produced (Schleifer *et al*., 1984; Jones and Ricke, 1994). Given the possibility that the organisms may not be distributed evenly in the feed, it is clear that it would be particularly difficult to take representative samples for analysis. In addition, the incidence of *Salmonella* varies from one kind of feed ingredient to another. Ingredients added in small quantities may be overlooked in any testing scheme, but could be critical if highly contaminated. Another factor that would increase the sampling requirement is that samples from the same batch of feed may need to be tested both before and after processing or pelletting.

When more data are available on feed as a source of pathogens, it may be possible to resolve some of these analytical issues and generate large databases. Some of the key factors and potential CCPs are becoming clearer, even from the limited data sets that have been reported. For example, Jones and Richardson (2004) noted that dust was a consistent source of *Salmonella* throughout the mill, and dust from some areas, such as pellet coolers, which draw in large volumes of air, was a major source of the pathogen. It is conceivable that dust in feedmills could be sampled as a measure of airborne *Salmonella* in the general environment. This might be associated with a particularly important CCP in the areas where pelleted feeds are stored, if there is a high risk of re-contamination. Thus, the sampling requirement discussed above may be reduced and the same approach has been applied in poultry rearing environments to relate airborne levels of *Salmonella* to bird colonization and litter contamination (Kwon *et al*., 2000a,b).

Numerous methods have been evaluated over the years for detecting and quantifying salmonellas in feeds and many have proved to be effective for these purposes with a variety of feeds (Williams, 1981b; Ricke *et al*., 1998). However, given the need for sensitivity, the use of molecular methods may be best in developing rapid, sensitive means of identifying small numbers of *Salmonella* in large volumes of feed. Several studies have involved polymerase chain reaction (PCR) assays and commercial kits to detect *Salmonella* in a wide variety of feed materials (Ricke *et al*., 1998; Maciorowski *et al*., 2000, 2005; Löfström *et al*., 2004). In addition, DNA array technology has been utilized recently to track the dissemination of a specific *Salmonella* serotype in Spanish feedmills (Alvarez *et al*., 2003). The main difficulty with routine use of molecular assays is the problem of extracting and recovering representative material from feeds for analysis (Macioroski *et al*., 2005). As more commercial assays become available, standard protocols for enrichment, extraction and PCR can be developed to determine the efficacy, detection limits and minimum enrichment time required in routine analyses of animal feeds.

Another potential solution to the problem of detecting low numbers of *Salmonella* in large batches of feed is to use an indicator organism or group of organisms that is consistent in its occurrence with the frequency of *Salmonella*-positive samples, but is present much more uniformly in feeds and in much higher numbers. One approach has used the detection of indigenous, male-specific and somatic bacteriophage that are specific for *E. coli* as a general indicator of fecal contamination (Maciorowski *et al*., 2001). Another approach involves enumeration of the family Enterobacteriaceae, which includes the genus *Salmonella*, and has been utilized for many years in food analysis (Mossel *et al*., 1963; Jones and Richardson, 2004), while also being suggested for use in examining animal feeds (van Schothorst and Oosterom, 1984; Veldman *et al*., 1995; Jones and Richardson, 2004). A correlation between Enterobacteriaceae and *Salmonella* has been observed for pelleted feeds (Stott *et al*., 1975). Likewise, in studies on feeds and environmental sites at a commercial feedmill, Jones and Richardson (2004) noted that counts of Enterobacteriaceae, although much higher than those of *Salmonella*, showed a consistent trend, being higher when samples were positive for *Salmonella* and lower in *Salmonella*-negative samples. Application of gene amplification and PCR detection of bacterial ribosomal genes have been examined as possible rapid assays for assessing the microbial quality of animal feeds (Maciorowski *et al.*, 1998, 2002).

7.5 Future trends

The main advances in improving the safety of poultry feed will probably come from the development of more sophisticated control measures. These are likely to involve better delivery systems for chemical disinfectants and more specific antimicrobial targets. Currently, the use of chemical disinfectants involves a broad application of the compound to the entire surface of the feed, which then serves as the delivery vehicle to the birds. However, as discussed previously, micro-encapsulation is now a feasible means of delivering organic acids and is likely to have greater application in the future. The issues that will need to be resolved include optimizing the encapsulation process so that the product remains functional in a typical feedmill and poultry house environment, and the economics of generating and applying the micro-capsules. Separation and containment approaches such as this also offer intriguing possibilities for using other, perhaps more potent or fragile antimicrobial substances that could be added to feeds and protected from the environment until they reach their target site in the bird.

The other anticipated advance is the development of feed antimicrobials that have more specific targets. One of the problems with most of the currently available chemicals is that they tend to have a broad spectrum of activity and not only are pathogens targeted, but also benign, indigenous organisms that might have served as a competitive barrier, either in the feed or the gastro-intestinal tract of the bird. However, there are several biological agents, such as

bacteriophage, bacteriocins and antibodies that can be produced to target specific pathogens. Usually, the main practical problems associated with these biological agents are cost and delivery. Consequently, protection by micro-encapsulation may be a useful means of avoiding the large amounts normally needed to ensure adequate treatment of the birds.

It is becoming more apparent that poultry feed needs to be included in overall surveillance programs that are used for foodborne pathogens in the USA. Although there are still practical limits to standardizing this approach across all feed operations, the limited data sets available indicate that there are some areas where good manufacturing practices would be effective in improving pathogen control, if more specific information were provided. For example, post-processing storage facilities in feedmills should be well separated from sites where raw materials are being received, and the two should not have a common ventilation system, but data to support these contentions are lacking at present. In future, more comprehensive sampling schemes and HACCP plans could begin with feed being produced for chicks, since these are highly susceptible to *Salmonella* infection, and such developments are likely to have a large impact on all downstream poultry production.

7.6 Sources of further information and advice

The strategies used for pathogen detection, surveillance and control in feed have been adapted largely from the literature on human food and foodborne disease. Therefore, most of the microbiology journals with an applied emphasis, and food science journals, are potential sources of information on analytical methods, etc. Also, the *Bacteriological Analytical Manual*, 8th edn, AOAC International, Gaithersburg, MD, is a source of standard methods for microbial isolation and enumeration.

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8

The effective control of *Salmonella* in Swedish poultry

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8.1 Introduction

Salmonella is one of the most important foodborne pathogens transmitted to humans from eggs and poultry meat (Thorns, 2000). Poultry flocks may be infected with *Salmonella* via hatching eggs, feed, water and from the environment, including birds, rodents, insects, etc. (Wray and Wray, 2002). In hens infected with invasive types of *Salmonella*, the bacteria may invade the developing egg and thus can be present in the egg contents. Faecal contamination of the eggshell may occur, when the egg is laid, and *Salmonella* present in the faeces may come to penetrate the shell. Poultry meat is usually contaminated at slaughter by bacteria from the skin or intestines of the live bird (Fries, 2002a). This chapter describes the *Salmonella* control measures that are applied throughout the poultry production chain in Sweden and considers the effectiveness of the strategies used.

8.2 Poultry production in Sweden

In 2003, 77 million broilers were slaughtered and 150 broiler-producers accounted for 99% of the production. The flock size varied between 1,500 and 45,000 birds, with an average of approximately 20,000 birds. About five or six flocks are produced in each unit annually. In 2003, the total number of laying hens was 4.5 million in 5,400 establishments. Among the producers, 200 had more than 5,000 birds, while 4,700 farmers had fewer than 50 birds. Production of turkeys, ducks and geese constitutes only a minor part of the total production of poultry.

8.3 The comprehensive control programme

In Sweden, a *Salmonella* control programme for poultry has been in place since 1970 and covers the entire production chain: poultry feed, breeders, hatchery, rearing, egg production, slaughter and processing. The programme includes laying hens, broilers, turkeys, ducks and geese from grandparent flocks to commercial stock, and consists of a mandatory and a voluntary part. The mandatory part involves the monitoring of all flocks, reporting of *Salmonella* isolations and eradication measures that are undertaken when positive flocks are detected. The voluntary part consists mainly of prophylactic hygiene measures and other steps to improve flock biosecurity. The control programme has been revised several times since the start, to accommodate new knowledge and changes in poultry production. During each revision, the industry has been involved in discussions with the authorities on which improvements in biosecurity to introduce and implement at any level of the production chain.

The objective of the control programme is to deliver *Salmonella*-free poultry meat and eggs to consumers. All serotypes of *Salmonella enterica* subsp. *enterica* are regarded as undesirable and all flocks found to be *Salmonella*-positive are destroyed. The policy is based on the fact that all serotypes are potentially pathogenic for humans, although some serotypes are more common than others (Wray and Wray, 2002). The decision to include all serotypes in the control programme has so far prevented the introduction and spread of new and potentially more pathogenic or invasive serotypes, and the global pandemics involving *S.* Enteritidis and *S.* Typhimurium DT 104 have not extended to Swedish poultry production. The low prevalence of *Salmonella* in poultry and other domestic animals in Sweden makes it feasible to include all serotypes in the programme.

In countries and regions with a higher prevalence of *Salmonella* in poultry, preventative measures, such as antibiotic treatment and/or vaccination, are used (Wray and Wray, 2002). These strategies have not been employed in Sweden, because the aim of the *Salmonella* control programme is to use other means of establishing freedom from infection in food-producing animals. By keeping such animals free from *Salmonella*, the number of animals that are carriers, and thereby may transfer the organisms to non-infected animals, is minimal. The practice of avoiding the use of antibiotics to treat *Salmonella* infections in Swedish animals has been shown to be beneficial in keeping antibiotic-resistant salmonellas at a low level (SVARM, 2003).

8.3.1 Concepts and strategies

The basic concept is that animals delivered for slaughter are free from *Salmonella,* following application of the following strategies:

- prevention of infection or contamination at all stages of the production chain
- monitoring of the production chain at critical points to detect any *Salmonella* contamination
- taking all necessary action to eliminate *Salmonella,* whenever the organism is detected in the production chain.

8.3.2 Preventative measures

So far, the measures taken in the poultry-production chain have been very successful in reaching the goal that meat and eggs for human consumption should be *Salmonella*-free (National Veterinary Institute, 2004). Stated simply, the Swedish strategy is to prevent *Salmonella* infection in poultry production by maintaining a high level of biosecurity and adequate hygiene control measures, as discussed later in this chapter.

As a complement to the mandatory disinfection of *Salmonella*-infected chicken houses, competitive exclusion treatment was used between 1981 and 1990 for three consecutive flocks in the same poultry house. This practice reduced the number of recurrent *Salmonella* infections in broiler flocks (Wierup *et al.*, 1992). However, the method has been discontinued, as the commercial treatment products are no longer available in Sweden, mainly because they are not well defined or sufficiently well validated to be licensed.

8.3.3 Monitoring of poultry for *Salmonella*

All commercial poultry flocks are monitored routinely for *Salmonella,* using a bacteriological culture method approved by the European Union (EU). Serological tests are not used, as the requirement to detect all *Salmonella* serotypes makes serology impractical. Moreover, non-invasive serotypes give a poor immune response, resulting in low levels of antibodies in serum and egg yolk (Barrow, 1992). Instead, faecal and tissue samples from dead birds are investigated in the monitoring programme. The sampling regimen is designed to detect *Salmonella* at a prevalence of 5% or more with a 95% confidence limit. The monitoring is conducted at the farm level for breeders, commercial layers and meat-producing birds, but also at the abattoir to confirm the earlier findings. Since the prevalence of *Salmonella* in eggs from infected flocks is very low, the monitoring of layers is performed only at farm level (Anon., 1995).

8.3.4 Action when *Salmonella* is detected in poultry flocks

It is compulsory to report any isolation of *Salmonella* from poultry, regardless of serotype, to the veterinary authorities, and all isolates must be sent to the National Veterinary Institute for typing and antimicrobial-resistance testing. All *Salmonella*-contaminated flocks, irrespective of serotype, are killed by euthanasia and deemed unfit for human consumption. The low prevalence of *Salmonella* in Swedish poultry makes this radical strategy possible.

8.4 Legal basis for the control of *Salmonella*

The control of *Salmonella* in animals and feed is covered by the legislation on zoonoses and feed. These includes several governmental regulations and guidelines. It is compulsory to report any *Salmonella* infection that is detected in

domestic animals and, in each case, prompt investigation must be instituted to eradicate the infection. The legislation gives the Board of Agriculture the power to issue relevant restriction orders to prevent the infection from spreading. The voluntary control of *Salmonella* in poultry flocks is also regulated and includes guidance on hygiene control measures in poultry houses, the handling of feed on the premises and sampling schedules (which are the same as those in the mandatory programme). In addition, there are full instructions regarding procedures that apply when *Salmonella* infections are found in domestic animals, as well as details of routines to be followed and documentation requirements. Finally, financial compensation for losses due to eradication of the flock, production losses and other costs related to cleaning and disinfection of the premises is provided by the government and/or private insurance.

8.5 Critical points in the supply chain

Identification of critical points throughout the entire production chain is essential for an effective surveillance and control system for *Salmonella*. In poultry production, the obvious risks involve drinking water and feed, breeding stock, husbandry practices (from hatcheries to the transport of birds to slaughter or eggs for consumption), slaughter operations and further handling of meat products and eggs. In the following, the most important critical points, according to Swedish experience, are discussed. The content of the text is illustrated in Fig. 8.1.

Salmonella-free feed and water must be guaranteed. Contamination of feed and/or water by *Salmonella* will lead to continuous exposure of the flock, as large volumes of both are consumed by the birds. A 'clean' water supply is ensured by using the same standards for water quality as those that apply to water for human consumption, and this is mandatory in Sweden. Theoretically, *Salmonella*-free feed can be obtained either by using clean raw materials in feed manufacture or by proper heat treatment of feed before delivery to the flock. In practice, it has been impossible to ensure that raw feed materials are free from *Salmonella*, so all poultry feed is heat-treated, using a pelletting process. The feed production chain, from raw materials to the finished product, and transportation of feed to the farm, requires a separate analysis of critical control points in order to ensure freedom from *Salmonella* (for a review, see Davies and Hinton, 2000).

The house/room and equipment where the birds are placed must initially be free from *Salmonella*, otherwise residual contamination from a previous flock may cause infection, as has been shown in many studies around the world (Larsen *et al.*, 1994; van de Giessen *et al.*, 1994; Davies and Wray, 1996; Bailey *et al.*, 2002). A *Salmonella*-free environment can be achieved by the use of an all-in, all-out stocking system, with thorough cleaning and disinfection between flocks. Special attention must be paid to the latter aspect, if a *Salmonella* infection was confirmed in the previous flock. However, it should not be ignored, even if the previous flock was sampled and found to be negative for *Salmonella*. Moreover, the house must be kept free from *Salmonella* during the

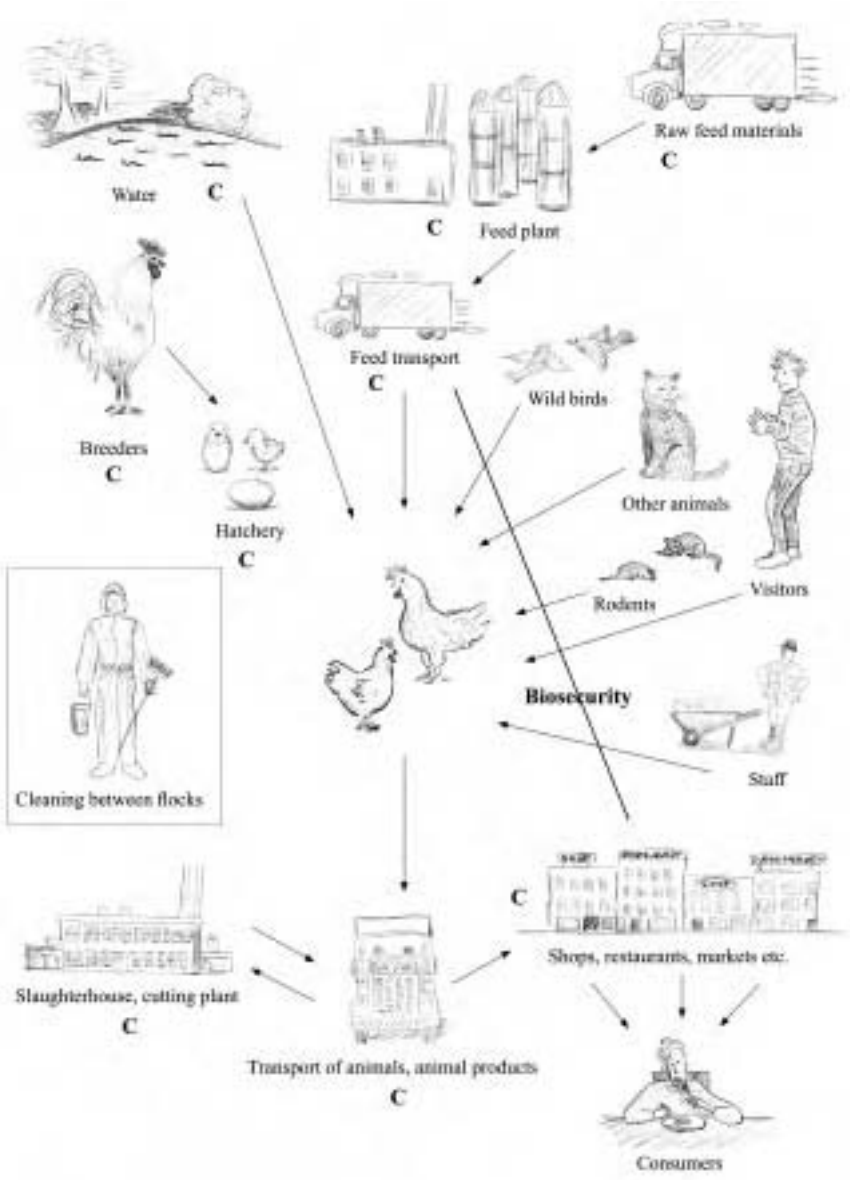

Fig. 8.1 Critical control points for *Salmonella* in poultry production. C = points for control sampling if relevant to the particular situation.

entire production period by applying good husbandry and biosecurity practices. Birds that are introduced into the clean environment must be *Salmonella*-free. This is ensured by strict control of breeding stock and hatcheries, and is discussed further below. Only eggs from well-controlled, *Salmonella*-free breeders should be used for hatching.

On all occasions that birds or eggs are transported, care must be taken to avoid introducing salmonellas into the transport vehicle during loading or unloading. Preventative measures include regular and thorough cleaning and disinfection of vehicles, transport crates, etc., but also the application of hygiene barriers and other hygiene measures during the handling of birds and eggs.

The entire slaughter process and further handling of the meat must ensure that no salmonellas are introduced at these stages and that no avoidable cross-contamination occurs at the abattoir or cutting plant. As mentioned already, the focus of the control programme is to deliver *Salmonella*-free birds to the abattoir. In consequence, there is no need to decontaminate the end product. According to the legislation, heat-treatment of any *Salmonella*-contaminated meat is allowed, but, so far, the domestic market has not accepted such products.

The Swedish experience has shown that implementation of preventative measures is of fundamental importance, but it is also vital to coordinate the sampling systematically, with the aim of being able to detect *Salmonella* at a certain prevalence (usually determined by practical and financial constraints), and to have an action plan in case *Salmonella* is detected at any point in the production chain. Unless appropriate action is taken when positive samples are detected, a favourable *Salmonella* situation can deteriorate rapidly. When *Salmonella* is detected, it must be possible to trace the infection/contamination throughout the entire production chain, in order to find the source and stop the spread of the organism.

8.6 Control of *Salmonella* in poultry feed

In the Swedish *Salmonella*-control programme for food-producing animals, the control of feed has proved to be an essential element. In accordance with the current legislation, all feed used must be negative for *Salmonella*. The system of feed control is based on the application of Hazard Analysis Critical Control Point (HACCP) principles throughout the production chain, and not on control of the end product.

Monitoring and control of feed in relation to *Salmonella* has been carried out by the feed industry since the late 1940s (Thal *et al.*, 1957). The reason for this early interest in *Salmonella* was not the organism *per se,* but the finding of *Bacillus anthracis* in imported meat-meal intended for pig feed. From this particular consignment, a large number of *Salmonella* serotypes was also isolated. Experiments using heat-pelletting were carried out in the mid-1950s, in an attempt to control *Salmonella* in feed ingredients (Swahn and Rutqvist, 1957). The need to develop procedures to control *Salmonella* in feed production became the primary objective of an industry association founded in 1958, comprising most of the Swedish feed companies. Several of the early guidelines on how to control *Salmonella*, particularly in broiler feed, were developed as industry recommendations in collaboration with government experts. Since 1972, all broiler feed has been heat-treated at a temperature above 75 ºC. From

1991 onwards, a HACCP approach has been employed in feedmills and is based on a risk analysis, with critical control points being monitored weekly.

In Sweden, the total volume of commercial feed for food-producing animals was approximately 2.2 million tonnes (metric tons) in 2003, of which 177,000 tonnes were broiler feed and 205,000 tonnes feed for layers. Currently, fifteen major feedmills produce approximately 95% of all feed consumed. About 70% of the mills are farmers' cooperatives and integrated systems of feed and animal production are not found.

By comparison with other food-producing animals, broilers and layers appear to be particularly prone to acquiring any *Salmonella* present in feed. In Sweden, there have been several examples of poultry flocks that were infected with *Salmonella* from feed, including: *S.* Infantis, *S.* Kentucky, *S.* Mbandaka, *S.* Newington, *S.* Liverpool, *S.* Livingstone, *S.* Tennessee and *S.* Senftenberg (Sandstedt *et al.*, 1980; Mårtensson *et al.*, 1984; Eld *et al.*, 1991; Häggblom and Aspan, 1997). Recently, new (i.e. not isolated previously in Sweden) serotypes have been detected in feed ingredients of plant origin. *S.* Yoruba, for example, was isolated from imported soybean meal in 1997. Since then, *S.* Yoruba has been isolated frequently from soybean meal and has also caused outbreaks in domestic pig and poultry operations. Data indicate a degree of resistance to high temperatures in this serotype (Häggblom *et al.*, 2002), which might explain the survival of the organism during pelletting of feed. The on-farm feeding of whole wheat to broilers was first permitted in 1995. Control plans and restrictions on the use of this material were introduced simultaneously. Epidemiological data indicates that, under Swedish conditions, the feeding of whole wheat does not present any increased risk for *Salmonella* infection of broilers.

8.6.1 Feed control measures

The control of *Salmonella* in commercial poultry feed is based on several strategies. An important part of the control programme is the HACCP-based process control system in the mill, where the main hazards are first identified in the processing line. The aim is to ensure that the processing line for poultry feed is not contaminated with *Salmonella*.

The most important risk factors in feed production are the raw materials. Previously, raw materials of animal origin were the most hazardous, but ingredients of plant origin are presently more important in this respect. In the control programme, quarantine and control of raw materials must be applied before ingredients are incorporated into compound feed, including domestic and imported products of both plant and animal origin. Domestic products are monitored by the supplier. All imported commodities are tested for *Salmonella*, either before or at the point of entry. The sampling protocol for raw feed materials is designed to detect *Salmonella* with 99% probability and an assumption that the organisms are evenly distributed in the material.

Soybean meal is often contaminated and approximately 30% of all consignments imported into Sweden are positive for *Salmonella*. Other high-risk

ingredients are rapeseed meal, corn gluten meal, fish meal and imported meat meal. Most salmonellas detected in such raw materials are so-called 'exotic' serotypes, i.e. not the types normally isolated from domestic animals. *S.* Typhimurium and *S.* Enteritidis are rarely isolated from feed ingredients. *Salmonella*-positive consignments of animal origin are rejected. Raw materials of plant origin that are contaminated with *Salmonella* are treated with organic acids, such as formic acid, before the feed is pelleted. The acid (1–2%) is mixed thoroughly with the contaminated ingredient in a mixer that is separated from the main feed production premises. After 48 hours, the treated material must be re-tested for *Salmonella* and, if negative, it can then be used in compound feed.

All poultry feed must be heat-treated at a minimum of 75 ºC, resulting in a reduction in *Salmonella* contamination of approximately 1000-fold. Inappropriate heat treatment during pelletting, condensation in the coolers and storage bins of the mill, re-contamination after heat treatment and poor cleaning of the premises are important risk factors. The hygiene of the premises is particularly important and efficient procedures for cleaning and disinfection must be implemented. After the heat treatment, care is taken to avoid re-contamination of the feed at the mill or during transport and storage at the farm level.

8.6.2 Monitoring of feedmills in Sweden

Weekly samples are collected from mills producing poultry feed and analysed for *Salmonella*. The samples are collected from the following points along the processing line:

1. the unloading pit for raw materials
2. the air aspiration filter
3. the top of the pellet cooler
4. the area surrounding the pellet cooler
5. the top of the bin for finished feed.

The samples collected are 'dust samples' or scrapings, often less than 25 g. End-point testing of finished feed is not carried out in the monitoring programme. Positive samples are most frequently obtained from sampling points (1) and (2), while *Salmonella* is rarely isolated after the heat treatment. In 2003, a total of 9,548 samples were taken in the HACCP control programmes of feedmills, 78 yielding *Salmonella* (National Veterinary Institute, 2004).

Action is always taken when *Salmonella* is detected in any of the samples from the monitoring programme. The precise action depends on the source of the positive sample(s) in the processing line, and will take place as soon as possible. Dry cleaning, followed by disinfection, is used in the normal sanitation programme, but ineffective disinfection may actually accentuate any *Salmonella* problems in feedmills. Approximately 30 samples (dust samples or scrapings) will be collected routinely from the processing line, mostly after the heat treatment step, and analysed for *Salmonella*. If all samples are negative, indicating that *Salmonella* is not present in the processing line, the investigation is

complete. If *Salmonella* is detected at the top of the pellet cooler, production will be stopped immediately and no delivery of feed is allowed from that production line. An extensive investigation will be carried out in the mill and including the batches of feed already delivered to farms. In Sweden, feedmills rarely have endemic *Salmonella* contamination, in contrast to crushing mills, which may be contaminated persistently.

8.7 Animal husbandry

8.7.1 Prevention of *Salmonella* infection at farm level

The basic principle is to introduce *Salmonella*-free poultry into clean poultry houses and to ensure that the flocks will remain *Salmonella*-free by preventing horizontal infection from feed, water and the environment. Under Swedish conditions, the use of a multi-layered control strategy will prevent the introduction of *Salmonella* into poultry flocks. Thus, an adequate biosecurity system at all levels is of paramount importance. The first requirement is an all-in, all-out stocking system for each poultry house that enables thorough cleaning and disinfection of the building and equipment to be carried out between flocks. Apart from this, it is also crucial that epidemiological units are correctly identified. This means that all units where poultry are kept are strictly separated with regard to direct or indirect contact with animals that can spread *Salmonella*. For example, manure, feathers, eggs, packaging material, tools, protective clothing and other materials that may be contaminated by faeces from one unit, must not come into contact with any other unit. For *Salmonella*, each compartment in a house may be considered an epidemiological unit if there are separate ventilation systems and there are no openings between the rooms. When this approach is followed strictly, it ensures that, in the case of a *Salmonella* infection in one unit, the infection is not spread to any other unit. Furthermore, the use of hygiene barriers around each epidemiological unit prevents *Salmonella* infection from possible environmental sources outside the building. For example, *Salmonella* has been isolated often from rodents (Jones *et al.*, 1991; Davies, 1995; Rose *et al.*, 1999; Davies and Breslin, 2001; Liebana *et al.*, 2003), wild birds (Köhler 1993; Cizek *et al.*, 1994; Davies and Breslin, 2001) and other wildlife (Davies and Breslin, 2001; Liebana *et al.*, 2003) outside poultry units. Workers, visitors and other people entering the poultry house have also been shown to be a risk factor for introducing *Salmonella* to poultry flocks (Fris and van den Bos, 1995; Lindqvist, 1999), and strict hygiene measures must be adopted in order to prevent such an occurrence. Visitors and workmen, for example, should not come into contact with the birds and this can be achieved by a well-planned system, where sensitive parts of machinery and equipment are placed outside the poultry house itself (Lindqvist, 1999). Included in the concept of a hygiene barrier is an ante-room that should be situated outside the room where the birds are kept. This room should be divided into two parts by a physical barrier, where the changing of footwear and protective clothing takes

place. The part of the ante-room that is closest to the external environment is considered to be potentially contaminated, while the part nearest the birds is regarded as the clean area. If several epidemiological units share the same ante-room, separate physical barriers must be installed at the entrance to each unit.

Poultry that are kept outdoors present a particular problem in relation to biosecurity, especially when it comes to maintaining adequate hygiene barriers, if the environment is contaminated with *Salmonella*. However, not all outdoor environments are contaminated with *Salmonella* and, with careful planning of location and husbandry practices, outdoor rearing will not automatically lead to *Salmonella* infection.

Many of the measures employed to avoid *Salmonella* contamination also prevent other zoonotic pathogens from entering poultry houses. For example, several of the hygiene measures recommended for *Salmonella* control have been shown to reduce the risk of *Campylobacter* infections (Berndtson *et al.*, 1996; Evans and Sayers, 2000; Gibbens *et al.*, 2001). The Swedish experience is that the biosecurity measures applied to control *Salmonella* have the benefit of limiting other infections that are spread by contact.

8.7.2 Prophylactic measures in the *Salmonella* control programme

In the voluntary control programme for Swedish poultry, the following requirements describe preventative and/or hygiene measures.

- Separate protective clothing and footwear must be worn by all individuals entering poultry units.
- In each house, there must be an ante-room for washing hands and changing clothing and footwear.
- The changing of footwear takes place at a physical barrier, which is also a seat.
- Experience suggests that the use of a footbath is not sufficient for decontamination purposes and therefore a change of footwear is required.
- Any tools and technical equipment may be brought into the poultry house only after thorough cleaning and disinfection.
- Only authorised personnel are allowed to enter the houses.
- At each farm, a professional rodent control programme must be established and all buildings should be constructed in a manner that prevents rodents and wild birds from gaining access. The preventative work includes sealing of holes and cracks where wild animals might enter the house, putting nets in front of windows and ventilation apertures, and keeping the area outside the buildings free from vegetation and rubbish.
- Spillage of feed around the silo may attract rodents and wild birds, and should be avoided. If it does occur, the spillage should be cleaned up immediately.

There should be a hard surface area that is easy to clean (e.g. concrete), immediately outside the doors, to prevent dirt being brought into the house. The house itself should be constructed to facilitate cleaning and disinfection, and all

materials used in the house must have smooth surfaces that are easy to clean. All drains and other outlets must be constructed to ensure that no back-flow of water, sewage, slurry or other material can occur. Litter materials to be used in the houses must be transported and stored in a manner that avoids contamination from wild animals or the environment.

In addition to the above, supplementary hygiene-control requirements for hatcheries, egg-packing rooms, etc., have been established. These follow the same principles as those used in the production process.

8.7.3 Regular sampling of flocks

In order to prevent any spread of *Salmonella* through the chain of production, and to optimise the control system, regular supervision and checks are important. The effectiveness of the control programme is supervised by official veterinarians and verified by regular sampling.

Within the mandatory *Salmonella*-control programme, regular sampling of all poultry flocks is carried out at each stage in the production chain, from grandparent to broiler flocks and commercial layers. The frequency of sampling varies with the different stages of the production chain and according to the impact that an undetected infection would have on the end-product. Breeding birds are sampled every month and every batch of newly-hatched chicks is sampled in the hatchery. Hens for commercial egg production are sampled twice during the rearing period, three times during the laying period and once before slaughter. Broilers, ratites, turkeys, ducks and geese are sampled at least once before slaughter, at the end of the rearing period. If *Salmonella* is detected at any point in the production chain, the result is reported to the authorities, who carry out investigations to determine the source of the infection. Between 1998 and 2003, 13 flocks of broilers and 26 flocks of layers were found to be positive for *Salmonella* (Table 8.1; National Veterinary Institute, 1999, 2000, 2001, 2002, 2003 and 2004, respectively). Among the layer flocks, there are several farms that have had a recurrent infection with the same serotype, indicating problems with the cleaning and disinfection of those farms.

Table 8.1 Number of *Salmonella*-positive broiler and layer flocks in Sweden, 1998–2003

Year	Broiler flocks	Layer flocks
1998	1	5
1999	4	5
2000	3	4
2001	3	5
2002	1	4
2003	1	3
Total	13	26

8.8 Breeder birds

Poultry breeding today is concentrated in a few breeding companies with worldwide markets. Most of these companies are concerned about *Salmonella* and keep their pedigree flocks free from all serotypes. In Sweden, all breeders are imported as grandparents and, to a minor extent, parents of broilers, layers, ducks and turkeys. They are all delivered as day-old birds and monitored intensively during the first month, when they are kept in isolation. *Salmonella*-infected flocks are killed by euthanasia, irrespective of serotype. Between 1982 and 1988, 12 out of 39 imported, broiler-breeder flocks were destroyed due to *Salmonella* infection (Wierup *et al.*, 1995). This policy has effectively minimised the introduction of new *Salmonella* infections from imported breeders. The risks of becoming infected with *Salmonella* are the same for breeders as for other types of poultry. However, since breeders are more valuable and may transmit the *Salmonella* to their progeny, stricter control measures are applied to breeders than production birds. The handling of hatching eggs is critical, because surface-contamination of the eggs may result in infected day-old birds.

8.8.1 Control of breeder flocks

The biosecurity measures for breeder flocks must be very strict. Each poultry house must be functionally isolated and separated from other houses. Rearing houses are easier to isolate than egg-production houses, as there are fewer movements in and out. Strict hygiene barriers should be applied to all breeder flocks, including staff being required to shower before entering the house. Staff should not be allowed to keep birds at home, and they should be in contact with as few flocks as possible on the farm. The handling of hatching eggs is critical. Roll-away nests in the breeder house are an important hygiene factor. Floor eggs or dirty eggs must not be used for hatching and slightly dirty eggs should be washed immediately after collection. The eggs should not be cleaned with sandpaper, because this may cause dirt to be rubbed into the pores of the shell. The hatching eggs should be sanitised as soon as possible after collection and stored in a dedicated room, with a separate entrance. When staff from the hatchery collect eggs, they must not come into contact with the birds. A register must be kept for each breeder flock, with details of egg production, bird health and deliveries of eggs to the hatchery, in order to facilitate the tracing of eggs, if a *Salmonella* infection is diagnosed in a flock.

8.9 Hatchery

All eggs must be sanitised with a suitable disinfectant upon arrival at the hatchery, initially in the incubators, when they are first set, and then on the day before hatching. In the hatchery, strict hygiene routines must be implemented. Visitors should be avoided if at all possible and a physical barrier placed at the staff entrance. The ventilation system should provide a flow of air that is in the

same direction as the movement of eggs between the different rooms, so that organisms from newly-hatched chicks cannot spread to the new eggs that have just been brought into the hatchery. Cleaning and disinfection routines must be efficient, transport crates returning from farms must be cleaned and disinfected in a separate room before being used for newly-hatched chicks, and a register must be kept of all hatches, delivery, etc., in order to be able to trace any salmonellas isolated from the offspring back to the parents. It is well known that good hygiene improves the hatchability of eggs and survival of chicks.

8.10 Dealing with infections in poultry houses

The action taken when an individual poultry flock is found to be positive for *Salmonella* appears to depend on the prevalence of *Salmonella* in the national flock as a whole. It is only practicable to adopt a policy of eliminating infected flocks if the prevalence is low and the cost can be balanced by the benefits. An elimination policy, with culling of all birds in the house and rapid sanitation of the premises, is beneficial for keeping the level of *Salmonella* contamination low in the external environment. If *Salmonella*-infected birds are loaded onto transporters at the farm and sent to the slaughterhouse, it is more difficult to avoid spreading the contamination.

Many studies have shown that *Salmonella* can be isolated from various sites in houses containing infected poultry flocks (Jones *et al.*, 1995; Davies and Wray, 1996; Hoover *et al.*, 1997; Davies and Breslin, 2001). Thorough cleaning and disinfection of the houses and all other potentially contaminated areas is essential to prevent infection of subsequent flocks (van de Giessen *et al.,* 1994; Larsen *et al.*, 1994; Davies and Wray, 1996; Rose *et al.*, 1999; Bailey *et al.*, 2002). Effective cleaning and disinfection require both scientific and practical knowledge of the critical points for pathogen survival, as well as systems in place to ensure that the work is performed according to instructions.

8.11 Dealing with *Salmonella*-infected flocks

Based on an already favourable *Salmonella* situation and a clearly defined strategy to keep the organism out of poultry meat and eggs, the mandatory *Salmonella*-control programme in Sweden includes a policy of eliminating all infected poultry flocks, irrespective of the serotype found. In this system, the farmers are compensated financially, when their flocks are destroyed, either by the government or by insurance cover. For layers, turkeys, geese, ostriches and ducks, the producer will receive governmental compensation at 50% of the market value of the birds and other costs caused by restriction orders or additional sanitation requirements, unless the farm is taking part in the voluntary *Salmonella*-control programme. For flocks affiliated to this programme, the compensation level is 70%. No compensation is paid for *Salmonella*-positive

broiler flocks, however, and all producers of broilers are required to have an insurance that covers most of the costs.

In the following, the cleaning and disinfection routines that are employed on poultry farms in the official control programme, are described. More detailed descriptions are given by Engvall (1993) and Svedberg (1993). After culling of the flock, carcasses are either buried on the farm or transported to a dedicated plant for destruction. Either way, carcass removal and transport from the houses must be done with as little spillage as possible, and in leak-proof transporters, in order to avoid environmental contamination. Intensified rodent control should be instigated immediately, to avoid rodent migration to other livestock operations, thereby spreading the infection.

All litter is removed and either buried, composted or spread and immediately ploughed into arable land. Spreading on pasture or grassland is not allowed. Potentially contaminated areas outside the buildings, e.g. where spillage from carcass- and litter-removal operations may have occurred, are disinfected with slaked lime or some other suitable disinfectant, after the removal of all visible spillage. In the case of heavy contamination, the surface layer is removed and replaced by new gravel. Any remaining feed is removed from the buildings and destroyed.

The first step in the cleaning process involves dismantling all removable equipment and dry cleaning (shovelling, sweeping, vacuum-cleaning) of all areas, including every item of equipment, as well as feeding and watering systems, roof and ventilation ducts. Among the means by which *Salmonella* can be transmitted are through air and dust (Holt *et al*., 1998; Davies and Breslin, 2001), and it is important that dust is removed. Old or damaged equipment that is difficult to clean should be discarded. In houses for commercial layers that are furnished with cages or high-density aviary systems, cleaning is difficult, because most of the equipment cannot be dismantled. When dry cleaning is completed, soaking, washing and rinsing are carried out. Hot or warm water is preferred, and detergents are useful, as well as pressure-spraying. However, when using high-pressure spraying, care should be taken not to create aerosols that could spread *Salmonella*. All work is done from top to bottom of the house to avoid re-contamination of areas already cleaned. After completing the wet cleaning stage and ensuring that all visible dirt has been removed, repairs to ensure even surfaces, as well as filling of cracks and holes where pests might enter the buildings, are undertaken. When repair work is complete and the building and equipment are visibly clean, disinfection, either by spraying or fogging, is carried out. It is important to follow the instructions of the disinfectant manufacturer. Improper mixing or application of disinfectants has been associated with failure to eliminate *Salmonella* from poultry houses (Davies and Wray, 1996), and low ambient temperatures are well known to be detrimental to disinfectant efficacy.

The disinfectant is left to dry and, after this, environmental samples are taken at critical control points for *Salmonella* culture. These must be negative before restrictions on the farm are lifted. In the case of positive environmental samples,

the entire house must be re-cleaned and disinfected again. In breeder farms, broiler chickens may be used as 'sentinel' birds to determine whether the poultry house is *Salmonella*-free, before any new breeder birds are introduced. During the entire cleaning and disinfection operation, hygiene barriers must be maintained between areas at different stages of the process, as well as around the entire farm. All personnel involved should have adequate information about the risks of *Salmonella* contamination and how to avoid them, as well as how to ensure personal safety and prevent infection while working on the premises.

8.12 Control of *Salmonella* at slaughter

If poultry infected with *Salmonella* should enter the slaughterhouse, there is a risk that the organisms will contaminate a large number of birds during processing (Hafez, 1997; Fries, 2002a,b; Olsen *et al.*, 2003). Apart from this, there is also a risk that the pathogen will become established in the slaughterhouse and on the equipment. Once inside the plant, *Salmonella* can spread easily and be isolated from various sites along the slaughter line (Morris and Wells, 1970; Olsen *et al.*, 2003). Morris and Wells (1970) found that *Salmonella* could be isolated more frequently from environmental samples in the slaughterhouse than from the carcasses. Even though the microbiological quality of poultry meat is largely determined by the corresponding status of the live birds, a great deal of attention must be paid to limiting cross-contamination throughout the slaughter process (Morris and Wells, 1970; Lillard, 1990; Fries, 2002a; Olsen *et al.*, 2003). In countries or regions with a prevalence of *Salmonella* that makes it difficult to avoid bringing *Salmonella*-infected birds into the slaughterhouse, flocks that are known to be *Salmonella*-positive can be slaughtered under strict hygiene precautions at the end of the day, followed by full cleaning and disinfection of the plant. This strategy reduces the risk of contaminating the plant and cross-contaminating other carcasses. However, Swedish experience shows that it is possible to avoid introducing *Salmonella*-positive flocks to the slaughterhouse, if control of *Salmonella* is effectively carried out at the farm level.

8.12.1 Sampling of slaughtered poultry

Five days prior to slaughter, all flocks are tested for *Salmonella* according to the monitoring programme and only negative flocks are allowed to be sent for slaughter. The *Salmonella* control programme in poultry meat establishments is based on bacteriological examination. At slaughter, neck-skin samples are collected in order to monitor the efficiency of the control programme at farm level and to confirm that there is no contamination in the slaughterhouse. Between 1998 and 2003, a total of 24,178 neck skins were analysed. Of those, only six samples were positive for *Salmonella* (Table 8.2; National Veterinary Institute, 1999, 2000, 2001, 2002, 2003 and 2004, respectively). These results

Table 8.2 Number of neck-skin samples taken for analysis at major slaughterhouses under the Swedish *Salmonella* control programme, 1998–2003

Year	Samples	Positive samples
1998	4 010	1
1999	3 580	2
2000	3 882	0
2001	4 184	0
2002	4 358	3
2003	4 164	0
Total	24 178	6

show that the strategy of controlling *Salmonella* at the farm level ensures *Salmonella*-free poultry products for human consumption. The frequency of sampling is based on the capacity of the establishment. From the major slaughterhouses (slaughtering more than 99% of all poultry in Sweden), two neck-skin samples are collected each day (morning and afternoon). Where positive results are obtained, the farm that yielded the positive birds is traced, additional sampling is carried out on the site and sampling is increased at the slaughterhouse. If *Salmonella* is isolated again, proper hygiene measures are instituted to eliminate the contamination.

In cutting plants, the bacteriological samples consist of macerated meat and trimmings, for example, samples taken from conveyer belts and tables. As in slaughterhouses, the frequency of sampling is based on the capacity of the establishment. When *Salmonella* is found in meat samples, the origin of the contamination must be traced back to the slaughterhouse or holding, whenever possible. Effective cleaning and disinfection of both the establishment and the equipment must begin immediately. Following confirmation of the result, an increased level of sampling is carried out during the next five working days.

8.13 Conclusions

There are many reasons for the favourable *Salmonella* situation in Sweden. Primarily, there was the fortunate timing of the introduction of feed control, political awareness of the human health risks and a cooperative climate between industry and authorities, when the control programme was instituted. When the programme first started, the structure of the poultry industry was different from that seen today, with smaller rearing houses of usually poor standard with respect to hygiene control. On implementing the *Salmonella*-control programme, these buildings were gradually replaced by better, more modern poultry houses, a fact that could have contributed to the reduction in *Salmonella* prevalence in poultry. Moreover, the domestic market did not favour the

importation of products that were possibly contaminated with *Salmonella*. The present international trade in animals, animal products and feed ingredients, coupled with the increased movement of people, provide new challenges in controlling infectious diseases.

It can be concluded that the comprehensive and co-ordinated *Salmonella*-control programme used in Sweden has worked out successfully. The control of imported breeders has effectively prevented the introduction of *Salmonella* into the Swedish poultry production chain, and *S.* Enteritidis was never introduced into Sweden. *Salmonella*-free feed was identified early on as one of the key factors in the control programme, and it is still one of the most important aspects, since raw feed materials are often *Salmonella*-positive. Keeping the feed free from *Salmonella* and applying strict biosecurity measures that prevent introduction of *Salmonella* from other sources has been shown to be effective in ensuring the production of *Salmonella*-free poultry. A continuous decline in the number of *Salmonella* isolations from poultry has been reported over the last few decades (Karlsson *et al.*, 1963, Hurvell *et al.*, 1969, Gunnarsson *et al.*, 1974, Sandstedt *et al.*, 1980, Mårtensson *et al.*, 1984, Eld *et al.*, 1991, Malmqvist *et al.*, 1995, Boqvist *et al.*, 2003).

The basic *Salmonella* control effort for poultry is carried out at farm level. The low level of *Salmonella* in slaughterhouses confirms the fact that *Salmonella*-positive flocks are being detected effectively before going for slaughter. An important aspect of the comprehensive control programme is the recognition that all *Salmonella* serotypes are equally undesirable. By using this approach, neither new variants nor well-known serotypes will be allowed to spread and become established in the food production chain. The present strategy has the aim of protecting the current situation and it would be much more difficult and costly to implement a control programme in a situation where *Salmonella* was more common.

8.14 Future trends

The Swedish *Salmonella* control programme for poultry is expensive to operate, but, at present, there is no inclination to change the current strategy. The general consensus within the EU is that foodborne salmonellosis needs to be controlled in order to reduce socio-economic costs and human suffering. To establish an optimal control strategy in different countries and regions, cost-benefit analyses should be performed regularly.

There has been a change of attitude in society in general with regard to foodborne infections and other hazards in the food chain. This change has led to increased awareness among consumers, international organisations and producers, which, in turn, has provoked the introduction of more regulations throughout the entire food chain. In the EU, new legislation on zoonoses has been introduced recently. Also, work in the Codex Alimentarius, Office Internationale des Epizooties and the World Health Organisation emphasises the

zoonotic aspects of animal infections and the need for effective means to control zoonotic agents in the food chain. The adoption of the precautionary principle leads to higher costs, when no scientific data are available. Thus, there is a need for more research in various areas of food safety. With regard to zoonotic infections in poultry production, more information is needed about the costs of human disease in relation to those of various control measures. The continuous change in the genome of zoonotic bacteria will result in new challenges in the future to prevent human disease and the occurrence of bacteria carrying, for instance, antibiotic-resistant genes that can spread rapidly worldwide. Studies of the pathogenicity, virulence factors, environmental persistence, antibiotic resistance, clonal spread and general epidemiology (in particular, biosecurity measures) are important areas for future research on zoonotic bacteria.

Consumer demand for safe food that is cheap, but of high quality, and produced to meet high standards of animal welfare are challenging goals for the food industry of the future. In order to ensure the continuing availability of safe food, many aspects of animal production need to be considered. Political aspects, human health, science and economics will need to be well balanced, when designing control programmes in the future.

8.15 References

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9

The use of probiotics to control foodborne pathogens in poultry

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9.1 Introduction

In the United Kingdom, the main foodborne pathogens associated with poultry are various serotypes of *Salmonella enterica*, *Campylobacter* spp. and *Clostridium perfringens*. These organisms have in common the fact that they rarely cause disease in the birds and are generally carried asymptomatically in the gastro-intestinal (GI) tract by a varying proportion of all healthy individuals. Their presence in the birds may lead to contamination of poultry products and therefore is a potential hazard to human health. However, when poultry are reared intensively in controlled-environment housing, flocks can be protected from extraneous pathogens by appropriate biosecurity measures. The term 'biosecurity' has been defined by Lister (2002) as 'a set of management practices which, when followed, collectively reduce the potential for the introduction or spread of disease-causing organisms onto and between sites'. The concept covers a range of activities, including maintenance of site security and environmental control, cleaning and disinfection of houses after use, implementation of national and international eradication measures, as well as the use of biological controls, such as vaccination and 'competitive exclusion' (CE) treatment (Nurmi and Rantala, 1973). More recently, effective biosecurity has become identified with the application of Hazard Analysis Critical Control Point principles to the overall production process.

Complete eradication of foodborne pathogens from commercial poultry flocks is an unrealistic goal at present. This is partly due to the complex epidemiology of flock infection in the case of *Salmonella* and *Campylobacter* spp. With these organisms, there is a variety of potential sources and routes of transmission that make overall control difficult (Poppé, 2000; Newell and Wagenaar, 2000; Cox *et*

al., 2002). Another important factor is cost, which limits the extent to which changes can be made in either premises or management practices. Therefore, significant improvements in pathogen control tend to occur relatively slowly, but it is common now to find national targets for specific pathogens. For example, in 2000, the UK Food Standards Agency set a target to reduce *Salmonella* contamination of retail chicken produced nationally by 50% within five years. Efforts involved in meeting this target have included the identification of necessary improvements in industry practices and, where possible, the development of new control measures. It is clear from the above that control of foodborne pathogens in live poultry is multifactorial, a situation in which the use of probiotics, as described in this chapter, can play an important part.

9.2 Native microflora of the alimentary tract

9.2.1 Advances in analytical methods

The GI tract of warm-blooded animals, including poultry, is a highly complex ecosystem in which there is considerable diversity among the microbial populations present. Understanding the nature of this microflora and its interactions with invading pathogens is a prerequisite for effective pathogen control by flora manipulation. In practice, our knowledge of the composition and activities of the native microflora is dependent on the suitability of available analytical methods. Early studies involved the use of selective isolation media and aerobic incubation, and would have greatly under-estimated the largely anaerobic populations of the lower bowel (Barrow, 1992). However, once the requirements for cultivating obligate anaerobes were properly understood, the necessary media and isolation techniques were soon developed. These were based on the roll-tube technique of Hungate (1950) and subsequent development of the anaerobic glove box. With these techniques, it became possible to carry out detailed analyses of gut microfloras and to isolate and characterise many of the organisms present, but such studies tended to be slow and laborious. Also, not all the organisms can be recovered on available isolation media, even when complex supplements, such as rumen fluid, are added (Salanitro *et al.*, 1974). Culture techniques pose other problems, too. While bacterial colonies growing in roll tubes can be picked for identification from the highest sample dilutions showing growth, organisms occurring at levels of one per cent or less of the total cultivable flora require the use of selective media. These only exist for certain bacterial groups. Once isolated, the obligate anaerobes are often difficult to purify, requiring several purification steps. Some of them survive poorly in culture media or change their morphology and those that stain Gram-positive in young cultures may rapidly become Gram-negative. Certain of the spore-forming organisms can be overlooked, because sporulation is often poor in culture media and heat resistance relatively low. Furthermore, phenotypic tests, such as sugar reactions, can give notoriously variable results. With all these constraints, even identifiable organisms can be misidentified but, for many of

the caecal anaerobes, it has not been possible to identify them with known species.

In addition to the above-mentioned technical difficulties, two major problems are evident in relation to analyses based on cultivation techniques. One is the inevitable bias towards isolating certain organisms and the other is the lack of a phylogenetically-based classification scheme for the organisms as a whole. However, modern molecular methods are overcoming these limitations and opening up new and exciting opportunities to unravel the complexity of the intestinal microbiota. An approach that is commonly used now involves the analysis of certain, carefully chosen gene sequences. Ribosomal RNA genes, for example, contain both highly conserved and relatively variable regions. The sequence changes that have occurred reflect microbial evolution. Short sequences (oligonucleotides) that are specific to bacterial phyla, genera or species can be selected. Through the specificity of DNA : DNA or DNA : RNA hybridisation reactions, selected oligonucleotides will recognise any desired phylogenetic grouping and, by suitable labelling, can be used as probes for enumerating particular groups in total nucleic acids extracted from samples of gut content. Use is made of the extensive databases that exist on known sequences as a means of determining the nature of the organisms present. Thus, the molecular approach is independent of cultivation methods. In fact, phylogenetic analyses based on 16S rRNA gene sequences that employ universal or more specific PCR primers have been used to study microbial communities in a number of habitats. These and other molecular analytical methods have been reviewed in relation to gut floras by McCartney (2002). A drawback of the PCR-based methods is that they, too, are liable to show inherent bias. Possible hazards include insufficient or preferential cell lysis, PCR inhibition, differential amplification and formation of chimeric or artefactual PCR products. However, various strategies have been developed to reduce such bias.

Whether cultivation or genetic methods are used for gut-flora analysis, they are all relatively tedious, costly and time-consuming, and therefore carried out sparingly. There is also a need for simple, more rapid means of detecting changes in flora composition that could be used for screening purposes. Such an approach was taken by Baquero *et al.* (1988), using microtopography of Gram-stained smears of human faeces. These workers studied the morphological diversity of the faecal flora and recognised 40 different bacterial morphotypes, sufficient to show when diversity was being reduced by, for example, antibiotic treatment of the host. Later, Meijer *et al.* (1991) used phase-contrast microscopy of faecal suspensions, linked to digital image analysis, for the same purpose. Subsequently, however, little or no use has been made of these simple methods of flora analysis, especially in relation to livestock.

9.2.2 Flora composition and properties

Much of what is known about the flora of the alimentary tract in poultry comes from studies on the chicken, and this is the only avian species for which detailed

information is currently available. To a varying extent, microbes are present throughout the tract, from mouth to colon, and their distribution reflects the physico-chemical environment in each region and the relatively rapid rate at which food passes through the digestive system. Initially, ingested food is stored for varying periods in the crop, which is colonised mainly by *Lactobacillus* spp., especially *L. salivarius*, and supports a lactic-acid fermentation. In this organ, the redox potential is too high for obligate anaerobes and conditions are unsuitable for most other organisms to become established. The inner surface of the crop is composed of stratified, squamous epithelium to which the lactobacilli adhere, and they form an almost complete layer, two or three cells deep, throughout the life of the bird. Adhesion is mediated by a carbohydrate-rich capsular layer (Fuller, 1975; Brooker and Fuller, 1975). Other organisms that occur in generally low numbers include *Escherichia coli*, enterococci and yeasts. These organisms are at least partly controlled by the large populations of resident lactobacilli, which have both bacteriostatic and bactericidal properties (Fuller, 1977).

Conditions in the proventriculus and gizzard are even less suitable for most microbes and survival is largely a matter of acid tolerance, with pH values ranging from 1 to 4. In spite of this, *Lactobacillus* populations up to 10^8/g of contents were reported by Smith (1965), with much lower numbers of *E. coli*, enterococci/streptococci and yeasts. Microbial populations also tend to be limited in the duodenum and the proximal part of the small intestine, where Salanitro *et al.* (1978) reported that *Streptococcus (Enterococcus), Staphylococcus, Lactobacillus* and *E. coli* were the predominant organisms. Populations of obligate anaerobes included anaerobic cocci, *Eubacterium, Propionibacterium, Clostridium, Gemmiger* and *Fusobacterium* spp., comprising 9–39% of the total number of strains isolated. The greatest diversity was observed in isolates from the duodenum. In the ileum, on the other hand, there is an unusual, filamentous organism resembling *Streptobacillus moniliformis*, which is attached to the mucosa of villi. At the point of attachment, the bacterial cells penetrate the epithelial surface (Pearson *et al.*, 1992) and may be involved in nutrient absorption. Salanitro *et al.* (1978) suggested that the obligate anaerobes in the small intestine may play a part in the digestive function, although their precise role, if any, remains obscure.

The caeca are two blind, tubular structures located at the junction of the small and large intestine. Only liquids and the finest food particles can enter the caeca and more solid material is physically excluded by a system of valves. Since the caecal contents are discharged infrequently, conditions in the caeca are relatively stable and therefore favourable for microbial colonisation, so that the ultimate populations are large and diverse. Bacteria are the predominant organisms present, especially obligate anaerobes, which occur in the lumen at up to 10^{11}/g wet weight. Facultative anaerobes occur at lower levels, but yeasts, moulds and protozoa are rarely found in significant numbers. The caecal mucosa is covered by an extensive layer of adherent bacteria (Fuller and Turvey, 1971). The problems inherent in the analysis of such a complex flora, which is thought

Table 9.1 Caecal microflora of broiler-age chickens analysed by cultural and molecular methods: some examples

Analytical method	Main groups	Reference
Anaerobic roll-tube	Anaerobic, Gram-positive cocci Bacteroidaceae *Eubacterium* spp.	Barnes *et al.* (1972), Barnes (1979)
Anaerobic roll-tube	*Propionibacterium* and *Eubacterium* spp., Bacteroidaceae, *Clostridium* spp.	Salanitro *et al.* (1978)
16 S rRNA sequences	Unidentified rods and cocci *Ruminococcus* spp. *Clostridium* spp.	Apajalahti *et al.* (2001)
16 S rRNA sequences	'*Cl. leptum* group' '*Sporomusa* group' '*Cl. coccoides* group' Enterobacteriaceae	Zhu *et al.* (2002)
16 S rRNA sequences	*Fusobacterium prausnitzii*-like organisms Butyrate producers	Gong *et al.* (2002)
16 S rRNA sequences	Clostridiaceae-related organisms *Fusobacterium*-related organisms	Lu *et al.* (2003)

to comprise at least 200 different types of bacteria, have been discussed above. Examples of results obtained by both cultural and molecular analytical methods are summarised in Table 9.1.

In some of the earlier studies, Dr Ella Barnes and her colleagues, working at the Food Research Institute, Norwich, UK, developed a collection of reference strains, representing the principal types of bacteria found in chicken caeca. Of 43 such strains, 23 were identified to genus level, but only 11 could be identified with known species and the remaining nine strains were unidentifiable (Mead, 1989). These limitations make it difficult to compare the data obtained by cultural techniques with those from molecular studies. However, some broad similarities are apparent, as well as obvious differences. For example, in culture-based studies, Bacteroidaceae featured prominently, but the organisms were less significant in the studies based on 16S rRNA analysis. Whether or not this is a result of methodological bias is presently unknown. In both types of study, differences were observed between individual birds of the same age that were reared under identical conditions (Salanitro *et al.*, 1978; Zhu *et al.*, 2002). These may be important in relation to the response of a flock to probiotic treatment. There were also indications of differences between the mucosal flora and that of the lumen; for example, Gong *et al.* (2002) reported that certain, unidentified butyrate-producing bacteria were found only in association with the mucosa. Although many of the organisms detected in chicken caeca have yet to be fully

identified, it is likely that some, at least, will prove to be unique to that particular habitat, while others may be common to many different animals (Zhu *et al.*, 2002).

The caecal microflora is clearly complex in composition and its role in host nutrition is still uncertain. The anaerobes present are predominantly fermentative, producing a range of volatile fatty acids as metabolic end-products, which are available to the host. Also, the common property of degrading uric acid, the main nitrogenous excretory product of the bird, provides a source of nitrogen to sustain the flora and a means of decomposing an insoluble waste product that enters the caeca via a back-flow of urine (Mead, 1989). The organisms studied so far have shown little capability for degrading complex molecules, such as cellulose. However, the nature of the diet can change the community structure of caecal bacteria (Apajalahti *et al.*, 2001) and possible effects are considered below.

9.2.3 Factors affecting flora composition

The distribution and activities of microbes that occur in the GI tract involve complex nutritional and physiological interactions among individual organisms and between the organism and the host. The nature and size of the microbial populations present are also influenced by various host factors, such as age, diet, disease status, immune response and responses to environmental stress. There are also possible effects of antimicrobials that may be used to enhance growth performance or for prophylactic or therapeutic purposes. Factors of particular importance in the present context are discussed below.

Bird age

Although the GI tract of the newly-hatched chick is usually sterile, bacterial populations begin to develop rapidly. The organisms originate from either the eggshell or the hatching environment and are readily ingested by the chicks. Populations in the caeca soon reach about 10^{10}/g, but initially comprise only a few bacterial types, including enterococci, coliforms, clostridia (especially *Cl. paraputrificum*) and aerobic spore-formers, such as *Bacillus coagulans*. Subsequently, caecal populations show distinct changes, with different groups of organisms predominating at different times, before climax communities are reached (Barnes *et al.*, 1972). It can take several weeks for *Bacteroides* and *Bifidobacterium* spp. to feature as major components of the caecal flora. The relatively slow rate at which the flora develops has been attributed to the conditions of modern poultry production in which chicks are hatched and reared initially in highly sanitised surroundings, lacking any contact with the parent birds (Nurmi and Rantala, 1973).

Diet

The diet of the host has an important influence on the composition, distribution and activities of microbial populations, especially in the anterior part of the alimentary tract (Smith, 1965). In the lower bowel, microbial communities may

be less affected because of prior nutrient digestion by the host and, with chicken caeca, the physical exclusion of larger food particles. The effects of diet are both direct and indirect. Feed includes compounds that provide a wide range of carbon and energy sources for direct utilisation by intestinal organisms. On the other hand, certain grain-based diets can modify crypt-villus architecture in the host and alter the mucin composition of the tract (Sharma *et al.*, 1995; Sharma and Schumacher, 1995). Such changes appear to be capable of reducing *Campylobacter* colonisation in chicks (Fernandez *et al.*, 2000). It has long been known that rye-based diets can induce bacterial overgrowth in the small intestine, a condition that is controllable by antibiotic supplementation (MacAuliffe and McGinnis, 1971). Also, high levels of wheat and barley in the diet have been associated with the appearance of necrotic enteritis in chickens, due to intestinal proliferation of *Cl. perfringens* (Kaldhusdal and Skjerve, 1996), although the mechanism of the effect remains to be clarified. More recently, there has been growing interest in the feeding of non-digestible carbohydrates to poultry as a means of stimulating growth of bacteria that are considered beneficial to the host. This aspect will be considered in Section 9.4.3.

Various antimicrobials are included in feed or water for therapeutic, prophylactic or performance-enhancing purposes, although their use for performance enhancement is diminishing in some parts of the world because of public health concerns. These substances will have differing effects in modifying flora composition, and can lead to the development of antibiotic resistance among pathogens such as *Salmonella* and *Campylobacter* spp. and also elements of the native flora (Report, 1998).

Conventional wisdom suggests that the environment in which birds are raised is at least as important as the feed in determining the microbial populations that exist within the alimentary tract. In addition, it has been apparent that relatively minor changes in feed composition, e.g. low to high protein content, have little effect on the microflora (Barnes *et al.*, 1972; Takahashi *et al.*, 1982). However, from a study involving molecular analytical techniques, Apajalahti *et al.*(2001) concluded that diet was a more important determinant of bacterial community structure in chicken caeca than farm management conditions, at least in Finland.

9.2.4 Natural mechanisms of pathogen control

Various factors are likely to determine the establishment of particular microbial strains at different sites in the alimentary tract and the formation of stable associations with other organisms in climax communities. These include environmental factors in the tract itself and others associated with the indigenous organisms. The regulatory processes will vary from one site to another and appear to be based on complex interactions that are still poorly understood. In this section, consideration will be given to microbial behaviour in the caeca of poultry, which are the principal colonisation sites for *Salmonella* and *Campylobacter.* The size and complexity of the overall microbial community present are significant factors in relation to pathogen control.

As mentioned previously, exclusion of invading pathogens that are not part of the native microflora depends upon the nature and degree of maturity of the microbial populations that occur. Possible control mechanisms include:

- competition between pathogen and native organisms for adhesion sites
- production of inhibitory metabolites by specific organisms
- competition for limiting nutrients
- establishment of environmental conditions that are inhibitory to the pathogen
- coaggregation between pathogen and certain native gut bacteria
- stimulation of the immune system.

Of these, competition for adhesion sites is considered important because adherent organisms are better able to become established and withstand movement of lumenal contents during digestion of food. This can involve an interaction between host cells and bacteria to form a glycocalyx, which is a network of long, charged polysaccharide fibres. Also, many bacteria are embedded in the mucus layer adjacent to the caecal mucosa of the chicken (Fuller and Turvey, 1971).

Among the possible inhibitory metabolites produced by native bacteria are lactic acid and volatile fatty acids (VFA) produced mainly by the obligate anaerobes. These include acetic, propionic and butyric acids, and are most active in the undissociated state below pH 6.0 (Table 9.2). Their effect in the gut is largely bacteriostatic and may be enhanced by low redox potential. A clear effect of microbial VFA on growth of *S.* Enteritidis under different conditions was demonstrated by van der Wielen *et al.* (2001, 2002). In young chicks, which have only a simple caecal microflora and are highly susceptible to *Salmonella* colonisation, Barnes *et al.* (1979) showed that the caecal pH was too high and the VFA concentration too low to inhibit colonisation by the pathogen.

Table 9.2 Bactericidal activity of volatile fatty acids in nutrient broth (based on Goepfert and Hicks, 1969)

Acid (0.5 %)	pH	*Log_{10} reduction in counts of *S.* Typhimurium			
		Contact period at 37 ºC (h)			
		8	12	24	48
Formic	5.5	1	3	4	4
	6.0	<1	1	1	2
Acetic	5.5	1	1	1	3
	6.0	<1	1	1	2
Propionic	5.5	1	1	2	4
	6.0	<1	1	1	2
Butyric	5.5	1	1	3	3
	6.0	0	0	0	2

* A 4-log reduction represented total destruction of salmonellas in the test suspension.
From Mead (1991).

Other metabolites that are known to have inhibitory properties include bacteriocins, which are produced by some strains of a given species and have a narrow spectrum of bactericidal activity, affecting only closely-related organisms. There are also substances of microbial origin with a wider range of antimicrobial activity, as well as free acids from microbial deconjugation of bile, and hydrogen sulphide. Some microbes can also produce appreciable amounts of hydrogen peroxide, but this is more likely to be significant in parts of the alimentary tract where oxygen is more abundant. The relative importance of these inhibitory metabolites in controlling enteric foodborne pathogens *in vivo* is not well understood.

Competition for limiting nutrients in the GI tract has been difficult to evaluate *in vivo* because of the physiological complexity of the ecosystem. In batch culture, Ha *et al.* (1994) showed that competition for serine was a critical factor in the growth of *S.* Typhimurium and *E. fergusonii.* Under anaerobic conditions, the salmonella was outgrown in serine-containing media and the effect was dependent on the redox potential. It was concluded that those organisms producing a low redox potential in the gut, which favours the strict anaerobes, may also provide an environment that is more restrictive to the growth of *Salmonella.*

Interbacterial adherence (coaggregation) increases the potential for intestinal colonisation by some lactobacilli (Vandevoorde *et al.*, 1992) and coaggregation between native gut bacteria and pathogens is a possible means of preventing pathogens from becoming established. By bringing the two types of organism into closer contact, it is also possible that this process enhances the effect of any inhibitory metabolites produced by native organisms. Although Jin *et al.* (1996) found little coaggregation between lactobacilli isolated from the chicken intestine and salmonellas, the general role of this phenomenon would appear to merit further study.

There is evidence that lactobacilli are among the bacteria that may play a part in the development of immune competence in young animals (Perdigon *et al.*, 1990). Also, Dunham *et al.* (1993) showed that birds treated with *L. reuteri* had longer ileal villi and deeper crypts, which is a response associated with enhanced T-cell activity and increased production of antibodies against *Salmonella*. Supplementation of layer rations with lactobacilli increased the cellularity of Peyer's patches in the ileum of the bird, thus indicating stimulation of the mucosal immune system (Nahashon *et al.*, 1994).

9.3 Use of probiotic organisms

9.3.1 Definition of the probiotic concept

It is well known that certain external factors, such as bird stress, dietary change or use of antimicrobials, can serve to disturb the natural balance of the intestinal microflora. Thus, benign or beneficial bacteria, including lactobacilli and bifidobacteria, may give way to increasing populations of potentially harmful organisms, such as clostridia or certain Enterobacteriaceae, or colonisation by

extraneous pathogens may be facilitated. One way of overcoming these problems is to administer beneficial organisms to the host via the oral route, using a microbial preparation known as a 'probiotic'. This has been defined by Fuller (1989) as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. The emphasis here is on the use of live organisms and, while the potential benefits of treatment may include improvements in bird health and growth performance, the aspect of interest in the present context is the ability of a probiotic to increase host resistance to colonisation by foodborne human pathogens that normally have no effect on bird health. Since poultry reared under commercial conditions are slow to develop a naturally protective gut flora, Nurmi and Rantala (1973) treated newly-hatched chicks with a saline suspension of material from the alimentary tract of *Salmonella*-free adult birds and showed that resistance to a subsequent *Salmonella* challenge was greatly increased through the early establishment of an adult-type microflora in the chicks. The protective process became known as 'competitive exclusion' or the 'Nurmi Concept', and various commercial preparations based on this principle are now available. However, they suffer from the disadvantage that their exact composition is unknown and therefore extensive testing is required to ensure freedom from specific pathogens and absence of any agents that might cause harmful effects on avian or human health. In practice, CE preparations have a long history of safe usage and are especially favoured in parts of Scandinavia, notably Finland and Sweden. Nevertheless, it is widely acknowledged that use of a defined microbial preparation would be preferable, if it was equally protective against pathogen colonisation.

Conventional probiotics, containing one or more microbial strains of known identity, have been marketed for many years for the treatment of livestock. The organisms used for this purpose usually include lactobacilli and certain bifidobacteria, which have 'Generally Recognised As Safe' (GRAS) status, and other microbial types, as appropriate. Examples of species incorporated in preparations used for poultry, together with specific chemical additives, are shown in Table 9.3. To qualify for use in a probiotic, each component organism should meet the following criteria (Fuller, 1992):

Table 9.3 Examples of microorganisms and non-microbial supplements used for probiotic purposes in poultry

Microorganisms	Non-microbial supplements
Lactobacillus acidophilus	
L. casei	Enzymes
L. reuteri	Electrolytes
L. salivarius	Organic acids
Enterococcus faecium	Vitamins
Aspergillus oryzae	
Saccharomyces cerevisiae	

- be suitable for the preparation of a viable product on a commercial scale
- remain viable and stable for long periods during storage and field use
- be able to survive, but not necessarily multiply, in the host intestine
- show a beneficial effect in the host.

The organisms should also meet certain safety criteria, both for use in poultry and in relation to personnel applying the product, e.g. absence of allergenic effects.

9.3.2 Selecting probiotic organisms

Essentially, the selection of microbial strains for probiotic use is made purely on an empirical basis, because the exact mechanisms by which they achieve their effects are uncertain. Candidate mechanisms for pathogen control were considered in Section 9.2.4. It should also be noted that beneficial effects associated with certain microbial species are not necessarily evident for all strains of those species and therefore extensive screening of strains is likely to be needed in order to select the organisms that are most effective for a particular purpose. Such screening will usually involve laboratory or pilot-scale trials but, ultimately, probiotics should be tested under field conditions to ensure that they have the desired effect(s) when used commercially.

Uncertainty about mode of action is due to the complexity of the intestinal ecosystem and the earlier lack of techniques by which administered organisms could be studied *in situ*. Modern molecular methods of flora analysis are likely to change this situation and allow *in vivo* behaviour to be assessed with much greater certainty. Until recently, strains were merely selected for their desired effects in the host, but other characteristics also need to be taken into account to ensure optimum performance. These have been described by Havenaar *et al.* (1992) and include tolerance to acid pH and bile, ability to adhere to intestinal cells, antimicrobial activity and stability during processing and storage. If the probiotic organism is required to colonise the host, then it is usually advisable to seek a strain from the same host species as that being treated, and even an organism from the specific site in the alimentary tract that needs to be colonised. Since not all probiotic organisms are required to colonise, any non-coloniser can be administered continuously via the feed. In this case, the origin of the strain is likely to be unimportant.

The search for organisms capable of inhibiting growth of foodborne pathogens in recipient animals is currently receiving much attention. The method used to screen candidate strains in the laboratory must ensure that inhibition is not merely due to acid production or catalase activity on artificial media, because neither property is likely to be relevant to conditions in the gut. The empirical nature of the selection process is highlighted by recent experience (USDA, 2004). In a study being carried out at the University of Arkansas, several promising probiotic combinations have been discovered, but, in doing so, more than four million isolates needed to be screened!

The use of relatively small numbers of microbial strains, which can be characterised in detail and tested for pathogenic and other possible harmful effects, such as the ability to depress growth-rate in the host, is an advantage in relation to probiotic development. Clearly, probiotic strains should be selected with care and fully identified before use. Strains of *Enterococcus* species are commonly used in probiotics and some are regarded as entirely safe, while others can be associated with disease conditions in poultry, e.g. certain strains of *Ent. durans, Ent. faecalis* and *Ent. hirae* (Smyth and McNamee, 2002). In addition, *Ent. hirae*, which was once classified with *Ent. faecium*, a species used in some probiotic preparations, can cause growth depression in young chicks (Houghton *et al.*, 1981). While probiotics of defined composition can readily be tested to establish their safety, this is more difficult for undefined preparations, comprising cultured gut content from a healthy donor bird in which many different microbial strains are likely to be present. However, such material can be screened comprehensively to ensure the absence of all known avian and human pathogens that are likely to occur, as well as organisms with acquired antimicrobial resistance. Since the material is cultured in a laboratory medium designed for the growth of anaerobic bacteria, there will be no multiplication of any viruses or protozoa that may be present, and these organisms should be eliminated by subculture during the manufacturing process. Some other possible pathogens, such as mycoplasmas, would grow poorly under the conditions of an anaerobic, mixed culture.

9.3.3 Methods of application

Within the European Union, probiotics of defined composition are regarded as feed additives, since most are incorporated in the feed that is given to recipient animals. As such, they are usually provided in powder or granular form for incorporation at the appropriate concentration. With undefined treatment preparations for *Salmonella* control, chicks were dosed initially by adding the material to the first drinking water. A disadvantage of this approach is that the birds often fail to drink before feeding, and the protective flora may not spread evenly throughout the flock. A better method, allowing chicks to be treated at the earliest possible opportunity, is to treat them by spray-application in the hatchery. While this can be done with a hand-held garden spray, it is preferable to use an automated spray-cabinet, enabling large numbers of chicks to be processed rapidly (Mead, 2000). Relatively large droplets are administered and the protective organisms are ingested as the birds preen themselves. The process may be enhanced by using a bright light that causes any droplets on the feathers to sparkle, thus attracting the birds' attention and encouraging preening.

9.4 Use of probiotics to control foodborne pathogens

9.4.1 Defined microbial preparations

Many studies have been carried out on defined microbial preparations to determine their potential for reducing or preventing intestinal colonisation of

poultry by foodborne pathogens. Most have used either *in vitro* model systems or small-scale laboratory trials involving chicks given an artificial, controlled challenge with the pathogen in question. Currently, there is little published data on the efficacy of such preparations when applied under commercial conditions, where a proportion of the birds may be already carrying the pathogen prior to treatment and subsequently the flock is exposed to a more variable and possibly repeated challenge. Moreover, the efficacy of the treatment under these conditions may be affected by various bird-management practices.

Early laboratory trials, reviewed by Mead and Impey (1987), showed that administration of lactobacilli, either alone or in simple mixtures with other organisms, to newly-hatched chicks had little effect on caecal carriage or shedding of salmonellas, following challenge. Various commercially-available probiotics were similarly ineffective in this respect (Hinton and Mead, 1991; Stavric *et al.*, 1992). However, more promising results have now been reported. For example, Morishita *et al.* (1997) have described what they term an 'avian-specific' probiotic, comprising *L. acidophilus* and *Strep. (Ent.) faecium*, that reduced by 70% the frequency of chicks shedding *C. jejuni.* Pascual *et al.* (1999) administered *L. salivarius* CTC 2197 to day-old chicks, together with a challenge dose of *S.* Enteritidis, and found that the pathogen had been completely eliminated by day 21, while challenged control chicks remained positive. Other lactobacilli would also be worthy of further study in this context. Unusually, *L. plantarum* is reported to possess mannose-sensitive receptors that may compete for the same intestinal adhesion sites as Gram-negative pathogens (Bengmark, 1998). Also, *L. reuteri* produces reuterin, which appears to be effective in chicks in reducing colonisation by *Salmonella* and *Campylobacter* (Mulder *et al.*, 1997). In another study, La Ragione *et al.* (2004) reported that *L. johnsonii* FI 9785 suppressed colonisation of chicks by *Cl. perfringens*, but had no effect on *S.* Enteritidis. Using *Saccharomyces boulardii*, a human probiotic, Line *et al.* (1998) reduced colonisation of broilers by *S.* Typhimurium from 70% in controls to only 5% in treated birds. Intensive screening of intestinal isolates, as reported by the USDA (2004), may yield organisms, or combinations of strains, that are even more effective against foodborne pathogens, but only when these have been evaluated under field conditions will their true potential for pathogen control become evident.

9.4.2 Undefined treatment preparations

Based on the original work of Nurmi and Rantala (1973), many studies have been carried out on the CE concept (reviewed by Mead, 2000). These have involved the use of suspensions or cultures of caecal content, scrapings of caecal mucosa, faeces or even litter on which chickens have been reared. In laboratory trials with one or other of these materials, chicks have been protected against colonisation by a variety of non-host-specific *Salmonella* serotypes and the treatment has also been used successfully in older birds, following antibiotic medication to clear an existing infection, as a method of restoring the normal

state of the adult gut microflora. It is apparent that protection against *Salmonella* by these means is independent of the breed, sex or immune status of the birds and it can be usefully combined with other *Salmonella* control measures, such as acid-treatment of feed and vaccination of the birds. Undefined treatment material includes a wide range of organisms capable of occupying the various ecological niches in the GI tract that would otherwise be available to salmonellas. In the experience of the author, the most protective CE preparation studied came from an adult bird that had been kept on five different farms, thereby maximising the opportunity to acquire a complex intestinal microflora. Generally, CE treatment can be expected to reduce significantly both the number of *Salmonella*-positive birds and the level of carriage in those that are colonised.

Treatment preparations that offer protection against *Salmonella* do not necessarily protect the birds against *Campylobacter* and, for those that do, culturing the material tends to reduce the protective capability. Susceptibility to *Salmonella* colonisation is greatest in very young birds due to the immature state of the gut flora, but this is not the case with *Campylobacter*, which readily colonises birds of any age, and suggests that a different protective principle is involved. Some broiler flocks appear to develop a naturally protective caecal flora against *Campylobacter*, but its nature is presently obscure (Mead, 2002).

Unlike the situation with 'conventional' probiotics, a number of field trials have been carried out on CE treatment, some of them on a very large scale. Initially, the treatment seemed relatively ineffective under these circumstances, due to various confounding factors, such as infection of chicks in the hatchery, prior to treatment. A more recent example of successful field use was the study by Derruyttere *et al.* (1997), involving more than a million broilers on 40 farms. When examined five to seven days before slaughter, none of the CE-treated flocks had acquired a *Salmonella* infection but, of the untreated flocks, 24% were *Salmonella*-positive. In this study, no *Salmonella* contamination was detected in the hatchery for either group of birds. The results suggest that effective use of CE treatment depends upon good biosecurity and, therefore, in itself, the treatment is not a 'magic bullet', but should be used as part of an overall *Salmonella* control programme.

Attempts to identify the key protective organisms in the donor flora led to the development of large treatment mixtures containing 48–50 separate organisms, including obligate anaerobes; these organisms represented the main elements of the culturable flora (Impey *et al.*, 1982; Stavric *et al.*, 1985). However, not only were they less protective in chicks than undefined preparations, but they tended to lose their protective capability during laboratory handling and storage.

9.4.3 Prebiotics and synbiotics

Prebiotics were defined by Gibson and Roberfroid (1995) as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health'. While this

definition was originally applied to substances given to humans, it applies equally well to animal diets. Since the host has little or no capacity to metabolise or absorb such substances, they pass virtually unchanged into the large intestine and are then available to the resident microflora. A particular prebiotic is chosen for its ability to serve as a substrate for one or more bacterial species with a potentially beneficial effect on the host and, by inference, it is not utilised by most of the other organisms present. According to van Immerseel *et al.* (2002), most of the prebiotics in current use are carbohydrates, including mono-, di-, oligo- and polysaccharides, which may be naturally occurring or synthetic.

One sugar in this category is lactose, a disaccharide, which meets part of the original definition of a prebiotic, because it is neither absorbed nor metabolised by the chicken and was shown by Corrier *et al.* (1993) to reduce both colonisation and organ invasion by *S.* Enteritidis. However, many of the native bacteria in chicken caeca can ferment lactose (Mead, 1989), so it would be unlikely to serve as a selective substrate. In this case, the anti-*Salmonella* effect is more likely to be due to overall stimulation of the flora, leading to increased concentrations of volatile fatty acids and a slight fall in caecal pH. In practice, lactose feeding of broilers has the disadvantage that it changes the consistency of caecal contents and causes mild scouring in the birds (Corrier *et al.,*1993).

The more complex fructo-oligosaccharides have also been studied in chickens in relation to pathogen control and shown to reduce intestinal colonisation by both *Salmonella* and *Campylobacter* (Fukata *et al.*, 1999; Schoeni and Wong, 1994). Mannose-oligosaccharides occur naturally in many plant materials, such as gums and yeast cell-walls. A commercially available product reduced caecal carriage of *Salmonella*, when given to broiler chicks (Spring *et al.*, 2000), as did a partially hydrolysed form of guar gum, included in the diet at a level of 0.025% (Ishihara *et al.*, 2000). In young hens, *S.* Enteritidis colonisation was eliminated from day 14 onwards by this means.

Ultimately, the effects of prebiotics against pathogens may be largely due to their influence on microbial metabolism, enhancing production of inhibitory metabolites. However, some can also bind to the cells of pathogens. The type 1 fimbriae found in organisms such as *Salmonella* bind specifically to mannose receptors and are needed for adhesion to the mucosal surface, thus facilitating colonisation. Indigestible carbohydrates with mannose residues may therefore bind to type 1 fimbriae and thereby prevent the pathogen from adhering to mucosal epithelium (Finucane *et al.*, 1999).

When a probiotic is administered, there is merit in providing the organism with a specific substrate for growth in the intestine. The combination of a probiotic and a prebiotic is known as a 'synbiotic' (Collins and Gibson, 1999). Synbiosis has been practised, with beneficial results, in relation to both defined and undefined probiotic preparations given to chickens (van Immerseel *et al.*, 2002).

9.5 Future trends

There is considerable interest in developing more effective measures to reduce the colonisation of poultry with foodborne pathogens and thereby diminish the risk to human health. For this purpose, the pursuit of ever-better probiotic organisms and combinations of strains is likely to continue, although, at present, strain selection can only be made empirically. Future developments in molecular methods of analysis that avoid analytical bias will improve understanding of flora composition and properties, and the *in vivo* roles of individual intestinal microbes. By elucidating the mechanisms of probiotic action and probiotic–host interactions, strain selection will become possible on a more scientific basis. In the longer term, the use of genetic engineering techniques will radically extend and even redefine the scope of probiotic action, as required, and the only apparent constraints will be in relation to environmental safety considerations and consumer acceptance.

At present, there is also a need for more work on prebiotics to determine the most effective and appropriate substances to be used as substrates for 'beneficial' components of the native flora or probiotic strains. It will also be necessary to investigate further the effects of prebiotics on the intestinal flora as a whole.

Whatever the means of developing probiotics, prebiotics or synbiotics, they must be tested in properly designed and adequately regulated field trials. Only then can their true value be assessed in relation to improving bird performance and reducing public health risks by controlling pathogen colonisation.

9.6 Sources of further information

A comprehensive account of the biological significance and importance of microbiota in the alimentary tract of a wide range of animals, including birds, is given in Mackie and White (1997) and Mackie *et al.* (1997). The use of probiotics, prebiotics and synbiotics for controlling *Salmonella* in poultry is discussed by van Immerseel *et al.* (2002). 'Competitive exclusion' and other possible means of manipulating the intestinal microflora are considered by Schneitz and Mead (2000). More general information on the control of foodborne pathogens in poultry flocks can be found in Mulder *et al.* (2002).

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10

The HACCP concept and its application in primary production

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10.1 Introduction

The Hazard Analysis Critical Control Point (HACCP) system, a means of managing risks in the food industry, has been around for several decades. Its success as a system has gained it both scientific and legislative recognition over the years, as it progressed from its rather specialised beginning in the space industry to the attempts made in many parts of the world to apply it throughout the food industry. Many have called for its introduction in all parts of the food chain, with slogans such as 'from farm to fork' and 'stable to table' becoming so commonplace as to be clichés. However, there are some difficulties in its implementation and even now, in the first decade of the twenty-first century, it is not applied universally in the mainstream food industry, even in countries where there is an encouragement or legal obligation to do so.

This chapter will start by exploring some of the above difficulties and why, in spite of them, we should be talking about applying the system in the primary production sector. It goes on to discuss in detail such topics as risks and hazards on the farm and how to deal with them in a risk-management and HACCP setting. Although this book concentrates on the poultry sector, it is considered more helpful in the present chapter to examine the broader field of primary production. Such an approach may show that, although there is always room for improvement, the poultry industry has already come a long way towards integrating itself into mainstream food production and processing. In addition, by taking a broader view, the advantages of implementing HACCP principles right across the primary production sector may also become more apparent. After many years of implementing, verifying, auditing and writing about HACCP in many countries, the author has become convinced that the

application of HACCP principles should start on the farm. It is to be hoped that, having read this chapter, most readers will agree with such a view.

10.2 Risk

It is essential to have a basic understanding of risk before discussing the HACCP concept and how to apply its principles. It is not intended here to discuss risk in detail – that would require a book on its own – but merely to highlight what the word means in the food safety context. People often talk of the HACCP system as a method of controlling hazards; strictly speaking it a method of controlling the risks that arise from those hazards. Risk can be defined as the probability of a hazard occurring, but that is only part of the story. A risk assessment looks not only at the probability of a hazard being present, but also at the severity of that hazard. Severity can easily be explained by using an analogy outside food safety. For example, it would be much more severe normally to be knocked down by a 26 tonne truck driving at full speed than it would be to be knocked down by a supermarket trolley. However, if you happen to be in a supermarket, the risk of being knocked down by the trolley is probably higher than that from the truck! Therefore, if we are to consider that being knocked down by a trolley in a supermarket is the hazard, then we cannot control that hazard – in effect it is always a hazard – but we can take measures to control the risk of it actually happening if, of course, we consider the severity to be great enough to justify any controls. In short, all of us, every day, are surrounded by hazards in any situation, but they are only of concern if the risk of their causing us harm is high enough for us to wish to eliminate or, at least, reduce that risk. This simple message about risk should be borne in mind as the chapter progresses.

10.3 Difficulties in implementing HACCP principles

A number of difficulties can arise when trying to implement and operate HACCP systems and, in general, these can be summarised as follows:

- Difficulties arising from bad communication.
- Difficulties arising from trying to use the system beyond its original design concept.
- Difficulties of over-complication.
- Difficulties with the basic requirements of good hygiene.

10.3.1 Bad communication

Most people learn about the HACCP concept from books, pamphlets, seminars, training workshops, enforcement officers or customers demanding that such a system be introduced. All these sources of information have several things in

common – they are usually contradictory, often ambiguous or confusing, seldom practical and, frequently, are almost completely unintelligible to the average worker in the food industry. In the following example, two different definitions of a hazard serve to illustrate most of these points:

> 'A hazard means the unacceptable contamination, unacceptable growth and/or unacceptable survival by micro-organisms of concern to safety or spoilage and/or the unacceptable production or persistence in foods of products of microbiological metabolism' (Pierson and Corlett, 1993).
>
> 'A hazard is anything – microbiological, chemical or physical – which may cause harm to the consumer' (MacDonald and Engel, 1996).

- *Contradictory* – The first definition talks only about microbiological criteria, whereas the second also includes chemical and physical criteria. In addition, the first definition includes spoilage! The first definition deals with 'hazard' from the viewpoint of the food and the second from that of the consumer of the food.
- *Ambiguous/confusing* – The first definition, as well as being over-long, includes two 'and/ors' and one 'or', making it the kind of sentence that would keep a bunch of lawyers happily and expensively in dispute for many years.
- *Impractical* – The first definition suggests so many possible variables that it makes a practical approach to dealing with the hazards all the more difficult to envisage, prove and implement. By contrast, the second definition, states clearly that, if it causes harm to the consumer, then it is a hazard and, by implication, the risk from it ought to be controlled – simple, straightforward and practical.
- *Unintelligible* – The average worker in the food and farming industries is not educated to university standard and yet definition number one contains phrases such as 'unacceptable contamination', 'unacceptable growth', 'unacceptable survival', 'micro-organisms of concern to safety', 'unacceptable production or persistence' and last, but not least, 'microbiological metabolism'. The lawyer for the defence would probably argue, with some justification, that this definition is written for scientists and not shop-floor workers in the food industry but, sadly, this, or similar, is often the definition used by trainers, when talking to shop-floor workers about the HACCP system.

This one simple example of the definition of 'hazard' is, unfortunately, not the only one that justifies the above comments. Even a cursory look at different training courses, syllabi, books, leaflets, conference papers, scientific papers, certified standards, etc., would make one wonder how any food company could even begin to implement a workable and effective HACCP system.

10.3.2 Beyond its original design concept

Leaving aside for the moment the romance of the space exploration industry, the original concept was designed for a linear-flow factory-type production model,

where relatively few raw materials are purchased, stored, prepared, processed, packed, stored and despatched. In this type of situation, flow charts are reasonably straightforward, Critical Control Points (CCPs) are easy to define and aspects such as monitoring and documentation can be kept to an effective minimum, while still fulfilling all the criteria of the HACCP system. Moving to the opposite end of the food supply spectrum, a fairly straightforward food processing system can be contrasted with a busy, city-centre restaurant, where the lunchtime menu has a choice of four starters, six main courses and four desserts and an à-la-carte menu of 60+ items; these menu items can have a vast number of ingredients, may be hot or cold, can be pre-prepared or prepared to order and with so many other possible permutations, it becomes almost impossible to list them. If one were to follow the Codex Alimentarius rules, it would not be long before the restaurant was full of flow charts, hazard analyses, risk assessments, monitoring documentation, CCP charts and bewildered restaurant staff, with no room left for either food or customers! In most countries with appropriate data, estimates usually suggest that about 95% of all food businesses fall into the category of non-manufacturing and many of these are catering businesses. It is understandable, therefore, that the HACCP system has received a lot of bad comments in the press. Can the sound principles of the original HACCP concept be applied in non-linear, non-factory situations? Of course they can, but it requires flexibility in both thought and action that, until now, has not always been apparent from either governments, industry or HACCP experts.

10.3.3 Over-complication

In the author's experience over many years, HACCP systems adopted by many companies are so complicated that they are both difficult to understand and expensive to operate. Why should they be so complicated? There may be many reasons, but the following are some that have been encountered, deduced or merely hypothesised about:

- *Consultants* – there is a role for consultants to play in implementing a HACCP plan, but most of the plan should be worked out by the 'HACCP team' of the food business. After all, they know their own business better than any outsider and, in addition, it gives them 'ownership' of the system. For many reasons, consultants tend to complicate HACCP systems, if they attempt to be solely responsible. For example, in the early 1990s, the author was asked to verify a HACCP system for a relatively simple confectionary product that was put in place by a consultancy company. A correct HACCP study may have identified two or three CCPs, but the consultants had identified 37 CCPs that, understandably, meant that the food company was having some difficulty in managing the system!
- *Due diligence* – many companies see their HACCP plan as part of their 'due diligence' defence under UK food legislation and therefore try to cover every

remote possibility. This may or may not strengthen their defence, but it often makes their HACCP system unwieldy, unworkable and cost-ineffective.

- *Litigation* – lawyers in certain countries have a reputation for being over-zealous in raising civil actions, so it is perhaps understandable that food businesses wish to protect themselves as far as possible and, consequently, end up with complicated HACCP systems. It is, however, debatable whether a complicated and cumbersome system gives any more protection than a simple and effective one.
- *Lack of understanding* – in general, bad training is responsible for lack of understanding. Many managers in the food industry are convinced, for example, that the more CCPs there are in a system, the more effective the system becomes. This is clearly nonsense, since the whole point of the HACCP system is to have 'the simplest possible controls where they matter the most' (Mitchell, 1995). In addition (see above), many believe that the HACCP system is a means of controlling hazards when, in fact, it is a means of controlling the risks from those hazards. Although, *prima facie*, this point may seem more of a semantic one, when it comes to understanding what the HACCP concept is trying to achieve and how an effective system might be designed, it does make a difference to the approach adopted.
- *Customer requirements* – with the advent of global hygiene standards and third-party auditing, customer requirements are perhaps less confusing, contradictory, expensive and time-consuming than they were previously. However, some food processors still have the burden of trying to satisfy the diverse standards of different customers. Often the understanding of HACCP principles by some of these customers is less than perfect and this can also lead to over-complicated and contradictory systems.
- *Combination with other systems* – it is quite common for a food company to combine its HACCP programme with various systems of the International Standards Organisation (ISO) being used at the same time, and often this works very well, since it makes some sense in terms of systems management. However, many companies try to fit their own in-house Quality Assurance and other systems into their HACCP plan and end up with oddities, such as Quality Control Points, Pest Control Points, Hygiene Control Points and so on. These additional points not only make the system more complicated but also, in the minds of the shop-floor staff, often serve to dilute the importance of the CCPs.

10.3.4 Basic requirements of good hygiene

Before a HACCP plan is even attempted, there are certain basic hygiene requirements or prerequisites that should be in place at the premises. These include:

- pest control
- cleaning and disinfection
- waste management

- personal hygiene
- staff training.

All too often companies attempt to implement HACCP systems in situations where some or all of the above are unsatisfactory. It is worth remembering, for example, that even the most perfect HACCP system imaginable would be worthless if crucial members of staff fail to wash their hands properly! These prerequisites will be discussed more fully later in the chapter with respect to farms.

10.4 Why should HACCP be applied to primary production?

Following the earlier remarks about the reasons why HACCP implementation has often proved to be difficult, especially when attempts are made to apply it outside its original design concept, one might be forgiven for asking 'why even attempt to apply the system to primary production?'

The author was born and brought up on a croft in the Highlands of Scotland. A croft is essentially a small farm but, more cynically, it is defined as 'a small area of agricultural land surrounded by legislation'. It would be easy to imagine the (mainly unprintable) comments of the author's late father had he been told to carry out a HACCP study on his crops and animals. To him and many farmers, both small and large, farming is basically a business of growing crops, breeding and rearing animals and selling them for the best possible prices. The farmer would never have considered himself to be a food business; that was quite clearly the province of the butcher, the supermarket and the local hotels! Times have changed, however, and there are a number of reasons why food safety considerations and, by extension, the HACCP system are relevant at the farm level:

- Some controls are more effective at farm level – microbiological controls can and should start on the farm. Although in many cases control can be achieved further along the food chain, would it not make more sense if this was carried out as a precaution rather than a necessity? Food processing plants should not be in the business of 'cleaning up' unnecessary microbiological contamination. Also, it would be unwise to think that microbiological problems are confined merely to products of animal origin; the past epidemiological history of, for example, salad vegetables is far from reassuring. It is true to say that controlling the survival and multiplication of microorganisms is primarily a matter for the factory, but the problems of initial contamination are mainly associated with the raw materials.
- Some controls are more cost-effective at farm level – most HACCP plans in food processing plants pay particular attention to the detection and removal of physical hazards; these are, after all, the only hazards that a consumer actually sees! The majority of these foreign bodies are introduced with the raw materials and, although contamination from within the factory is, of

course, also possible, it is often more cost-effective to remove many of these hazards before they leave the farm, rather than trying to deal with them on a factory production line.

- Some controls are only possible at farm level – most chemical hazards arrive in the raw materials and, once a chemical hazard is present in a food, it is always difficult and usually impossible to remove it. Examples of these are residues of veterinary drugs and pesticides, both of which can be controlled effectively on the farm.
- Some controls are relatively easy at farm level – it is, for example, reasonably straightforward to reduce the microbiological load in milk, or to eliminate the risk of antibiotic residues in meat or milk, or to make use of potable water supplies for cleaning and irrigation purposes.
- There is evidence of growing public concern about food safety on the farm – it is hardly necessary to explain this heading, as one need only whisper such topics as BSE, nvCJD, *Salmonella* in poultry and eggs, Foot and Mouth disease, *Escherichia coli* O157, Asian bird flu and intensive farming to raise loud storms of protest from any group of Western consumers or media representatives. There is a growing desire among consumers for safe and ethical farming.

10.5 Key food hazards and risks on the farm

When discussing hazards in connection with the HACCP concept, it is common practice to deal with them under three headings:

- biological (usually more narrowly defined as microbiological)
- chemical
- physical.

However, most industry food standards now include two other categories and these will also be looked at briefly in this section:

- genetically modified organisms (GMOs)
- allergens.

This fairly straightforward grouping is largely self-explanatory, although it will be discussed in more detail below. Mycotoxins, toxic substances produced by moulds, are sometimes listed as biological hazards and other times as chemical hazards. For most practical purposes, the distinction has no real significance but, since they are normally considered to be adulterants, they will be discussed under the heading ‘chemical hazards’.

10.5.1 Biological hazards

The sources of biological hazards on the farm are almost without limit. Pathogenic bacteria, bacterial spores, protozoa, parasites, viruses, moulds, yeasts

and enzymes can be present in abundance and, depending on the type, can be found in the air, water, vegetation, soil, waste products, feeding materials, pests, domestic animals, livestock and humans. With such a matrix of possible hazards and their sources, how can one begin to make any sense of controlling the risk?

A good start is to know which organisms are associated with which particular sources. Time, energy and money are all precious commodities and there is little point in wasting them by trying to eliminate, for example, a hazard from water that is not normally present. There are more than enough hazards associated with, or transmitted by, each of the above sources, without trying to deal with extra ones unless a risk assessment shows that such an approach is necessary in a particular case.

It is worthwhile to consider water in more detail. After all, it is used in one way or another in every type of primary production. Typical organisms transmitted by water include:

- *Campylobacter jejuni*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*
- *Giardia lamblia*
- Hepatitis A virus
- Norwalk virus
- Rotaviruses
- *Salmonella*
- *Shigella* species
- *Vibrio cholerae*
- *Vibrio parahaemolyticus*

One of the dangers of writing lists is that there are always those who, often with strong justification, may argue for the inclusion or exclusion of certain elements. For example, this list does not mean that *E. coli* O157 cannot be found in water or is transmitted by it – indeed many would argue that it frequently is – but water is not normally regarded as an important source or vehicle of this pathogen. However, the following should show that the inclusion or non-inclusion of a particular organism is not of major importance.

Water is used in primary production for many purposes including:

- irrigation
- drinking
- as an addition to animal feeds
- for cleaning equipment
- for cleaning buildings and their environs
- for washing vegetables and fruit
- cooling
- heating
- ice making
- fire fighting.

With such a list, it is not difficult to decide which of these uses can directly or indirectly affect crops or animals. If, for example, the irrigation water is contaminated, it can directly contaminate the surfaces of fruit or vegetables, which, in turn, can infect the consumer. There are many recorded cases of human infection from salad vegetables that have been traced back directly to contaminated irrigation water. For the sake of simplicity, the term 'product' is used here for all cereals, vegetables, fruits, milk and animals destined for human consumption. How can we prevent the water from contaminating the product? First we need to answer a series of questions. These would include the following:

- How often is the water tested?
- What tests are carried out?
- Who is doing the testing?
- Will these tests indicate the possibility of any of the above hazards being present?
- Is there a history of problems with protozoa in the area?
- Is the water tested for the presence of protozoa?
- If it is a public supply, does the user have access to the test results?
- If it is a private supply, how effectively is the source protected from environmental contamination?
- What type of water treatment, if any, is used?
- How is this treatment monitored and documented?
- How often are the storage tanks cleaned out?
- Are the storage tanks protected from environmental contamination and access by pests?
- How often and how effectively are water dispensers for animals cleaned and disinfected?

If the answers to these questions suggest that the risk from all the hazards associated with water are not only under control, but also monitored well enough and frequently enough to guarantee such control, then there will be no problem from biological contamination of the water supply. In short, the use of uncontaminated water has become a CCP and, as a result, has prevented contamination from this source. Therefore, there can be no concern about the water being given to the animals, used to clean equipment, added to feed or employed to cool milk or irrigate crops.

Using a similar approach it is relatively straightforward to deal with all the above sources of contamination. In other words, it is neither practical nor cost-effective to try dealing with each individual hazard. The most effective action is to look at the source of the hazard(s) and try to reduce the risk at source. In general, if the risk from one pathogenic bacterium is reduced to an acceptable level, then the risk from *all* species of pathogenic bacteria is reduced to the same extent.

10.5.2 Chemical hazards

The possibilities for chemical contamination at the primary production level are just about as diverse as those for biological contamination. For simplicity, these

can be divided into three categories:

- contamination from chemical pollutants
- contamination from naturally occurring chemicals
- contamination from chemicals that have been introduced.

Chemical pollutants

Industry has left its unwelcome calling card on agricultural land throughout the world. Sometimes, this involves direct contamination of the soil, but often the contamination is indirect, as a result of polluted water or air. It would be naive to blame all of this on the Industrial Revolution of the nineteenth century and such problems continue to this day in every country where industry produces toxic waste products. Mining areas also provide their fair share of pollutants from waste disposal and water contamination.

More worrying for many people is the effect of military pollution on agricultural land. Independent scientific surveillance has shown that countries experiencing recent military conflict often have localised evidence of the effect, for example, of using depleted uranium in ammunition. The continuing blanket denials from various military authorities do little to reassure researchers, farmers and health authorities of, for example, the Balkans where kick-starting a slumped agricultural economy has proved difficult enough, without the additional worry of residual pollution. That from the nuclear industry must also be considered. Without becoming too involved in the politics of nuclear waste and the arguments and counter-arguments that currently rage across Europe, one need only quote the Chernobyl disaster of 1986 to illustrate the point that a significant part of the farming community across Europe is still affected by the pollution from this catastrophic event.

What sort of pollutants fall into this group? Mostly, it is heavy metals or radioactive waste. These prove extremely difficult, perhaps impossible, to eliminate and often the only answer is to avoid growing food for human or animal consumption in areas where there is a significant problem of this kind. Of course, some of these pollutants can be removed by tackling the source. For example, pollution caused by lead pipes or zinc tanks can be avoided simply by replacing the lead or zinc with non-pollutant materials or by coating them so that the metal does not come into contact with the water. However, it is worth mentioning that dealing with contaminated land is a job for experts.

Contamination from naturally occurring chemicals

The composition of the soil varies from place to place even over short distances. This is dependent on a number of factors, including the underlying geological structure, past geological events, the water table, predominant weather conditions, application of fertilisers, type of farming, height above sea level, distance from the sea and so on. Sometimes the presence or absence of a chemical compound or mineral can cause physiological changes in animals or plants. Having an accurate soil-survey report can help the farmer to make

decisions about which crops can be grown safely or what kind of intervention is available to change the composition of the soil to one that is more favourable to his requirements.

Chemicals that have been introduced
Chemicals can be introduced onto the farm in many ways and these can be summarised as follows:

- Those that are used to promote growth or increase yields, such as animal feed supplements, chemical fertilisers, veterinary drugs/preparations, pesticides and herbicides.
- Those that prevent or cure disease, such as veterinary drugs/preparations, pesticides, herbicides and cleaning/disinfection materials.
- Those that are used for cleaning or disinfection.

It is important to emphasise that these elements are controllable. The farmer/primary producer introduces these materials onto the farm as a deliberate act and therefore has complete control over, for example:

- what is bought
- why it is bought
- where it is bought
- what its ingredients are
- what alternatives may be used
- where it is used
- how it is used
- what withdrawal times are required
- what records are kept.

10.5.3 Physical hazards

Although self evident, it is perhaps worth pointing out that any physical food hazard that exists on a farm can also end up in the product leaving the farm and therefore, potentially, in food. The list could be almost never ending, and, of course, would depend on the product, but could include sticks, stones, insects, small animals, bones, faeces, earth, plant material, metal, and so on.

10.6 Genetically modified organisms (GMOs)

There are those who would, with some justification, query why this is listed as a 'hazard'. However, most major retailers, in their buyers' specifications and industry hygiene standards, insist upon either the absence of GMOs or that any present, even in trace amounts, are suitably notified on product labels. This can cause a problem, however, for cereal farmers that are adjacent to a 'licensed' GMO cereal farm. It can also pose a problem in proving that animal feed is free from GMO products.

For any who think that this may be far-fetched, the following short anecdote may serve to illustrate the current paranoia in the food retail industry about GMOs. One of the many uses of starch powder is as a binder for the inks in offset printing. Starch powder comes, in the main, from maize, which in mainland Europe, is so extensively subjected to GMO experimentation that it is often difficult or impossible to separate GMO maize from natural maize. Offset printing is normally used in the food packaging industry for secondary packaging, which never actually comes into contact with the food itself. Some food manufacturers insist that all food packaging materials, including printing materials, are guaranteed GMO-free. Therefore, the starch made from maize must be shown to be free from GMO material!

10.7 Allergens

The number of people suffering from allergies, allergic reactions and food intolerance seems to be on an exponential growth curve. It is not the purpose here to discuss this phenomenon and its causes and effects, but simply to point out that primary producers should be aware of the effects that their products can have on those who suffer from such ailments. It is should be easy for a peanut farmer to deduce that his product is itself an allergen, but what of the poultry producer who feeds his chickens with a pathogen-free, chemical hazard-free, certified, guaranteed feedstuff? Can he be sure that the feed is free from allergens and, if not, can he guarantee that these allergens will not be passed along the food chain to the human consumer of the poultry meat?

10.8 HACCP in a farm setting

In most countries, the majority of the population live in large towns and cities and their vision of the farming/country community is formed either by what they observe from a speeding train or a car on a busy motorway, as they make their way from one conurbation to another, or by what they see on television or read in a newspaper or book. What they often see is a pleasant picture of rolling countryside, comprising multi-coloured fields with, to them, often unidentifiable crops, areas of plain earth, farm animals grazing in lush grass and all of this dotted with ponds, rivers, unidentifiable farm buildings, country cottages, small rustic villages and people in funny hats driving tractors. However, the reality is that farming is a harsh, tough, 365-day per year job, from which the financial benefits are often meagre. Therefore, any attempts by the farmer to gain fiscal improvement ought at least to be met with understanding, if not actual outright approval. At the same time, of course, the farmer should be fully aware that what he produces is not simply a crop, but a human food, or an ingredient for human food, or an essential part of the food chain, and should carry out his business with that fact indelibly engraved in his thought and work patterns.

How can HACCP help to change the image of the farmer to one of a caring, careful, knowledgeable, consumer-orientated food producer? The answer is unlikely to be found in producing flow charts of the production of barley, hens' eggs or lean pork, or by dogmatically introducing targets or tolerances. Instead, the farmer needs to be aware of HACCP principles and apply them wherever they are appropriate, simple to introduce and operate, and where they make a measurable improvement in the safety of the product.

There are certain elements that could be termed HACCP prerequisites, Good Hygiene Practice or even Good Manufacturing Practice, and there is little excuse for not introducing them, at least to some extent, into the farming environment:

- pest control
- cleaning and disinfection
- waste management
- personal hygiene
- staff training.

It is pertinent to consider each of these in turn and examine what, if any, difference they would make to the safety of primary products.

10.8.1 Pest control

The Oxford English Dictionary defines a pest as 'a destructive insect or other animal which attacks crops, food or livestock'. Using that definition it would be easy to argue that no existing farm could genuinely be described as '100% pest-free'. However, is it essential that the farm should be entirely free from pests? In the following, some groups of pests are described and the risks that they pose from a food safety point of view are discussed.

Pests of growing crops

These are usually an economic problem, but are rarely a food safety issue *per se*, although their treatment with pesticide may well prove to be a food safety problem. History has shown that bird faeces can contaminate crops or water supplies, but the control of birds in open fields is not an easy matter. For example, both the 'townie' concept of a scarecrow and the more technologically advanced sonic devices now available have only short-term merits, if any, since birds adapt to them fairly quickly. The variety of pests can be extensive. In addition to birds, insects, rats, mice, rabbits, hares and deer, there is the occasional trespassing horse or livestock belonging to a neighbouring farmer. When decisions are made to deal with these pests, a risk assessment should be carried out to ensure that food safety is not being compromised by the proposed control methods.

Storage pests

This mostly applies to insects of various species and again, from a food safety point of view, the treatment is often more of a problem than the pest, although

such pests can give rise to physical hazards in the food. Birds can be a problem, but denying them access to the store is usually only a matter of good design and maintenance. Rats and mice are often present on farms and, without question, can be a human health problem, both from the point of view of contamination with pathogens and physical contamination from faeces or body parts, such as hairs. Integrated control of rodents, using both good husbandry and safe pest management, is relatively easy and only requires the advice and assistance of a pest control expert.

Pests associated with animals
There are many pests, such as fleas, that are associated with animals, but few of them are significant in relation to food safety, although, as above, their control may introduce hazards and risks.

In summary, pests that are of food safety significance should be controlled as far as is practically possible, but not by compromising food safety in other ways or by damaging the environment.

10.8.2 Cleaning and disinfection

It would be a mistake to think that cleaning and disinfection is only of importance to, say, the dairy farmer. Regular, thorough cleaning and disinfection of equipment and buildings should be a priority for every farmer, and helps with the control of microbial pathogens, as well as pests and physical hazards.

10.8.3 Waste management

Although a farm concentrating on animals or animal products will produce much more waste than, for example, a fruit farm, it is essential that every farm has a proper control and disposal system for waste. This is imperative, not only in relation to food safety, but also from an animal health perspective. Government agricultural departments issue free advisory leaflets on the safe storage and disposal of waste products, and the advice should be followed strictly.

10.8.4 Personal hygiene

Although it is not the norm for clean protective clothing to be worn regularly by farm workers or for 'Now wash your hands' notices to be displayed in every farm toilet, it is, or should be, a normal part of daily working life for those such as dairy farmers. While no-one would expect a person ploughing a field to wear a clean white overall, there are situations on a farm that may warrant more than a passing thought about personal hygiene. Would a customer be happy to know that the apple they are eating was packed in its box by someone suffering from diarrhoea, who washed his hands only rarely?

10.8.5 Staff training

It appears somewhat incongruous that the law in Europe should expect training in food hygiene for those who wash pots and pans in hotel kitchens, but not for others who produce the food or raw materials in the first place. A simple course in food hygiene, designed specially for primary producers, would do much to improve their knowledge and the confidence of the consumer.

10.8.6 Other on-farm factors and their control

What then of the classic HACCP concept of hazard analysis, risk assessments, flow charts, targets, tolerances, monitoring and so on? Does it have a role to play on the farm? In theory, the answer appears generally to be 'yes' but, in practice, generally 'no'. For example, there is no reason why the production of barley should not be reduced to a flow chart annotated with all the various pieces of information that would go to make up a HACCP plan. In practice, however, would this actually improve food safety? The average farmer would have many such flow charts to construct and, very possibly, would become frustrated and tired at having to document and record things that either he sees as unnecessary or does anyway in a different manner. Earlier, the use of water and its control on the farm were discussed. By controlling the water supply, the risk from many hazards in different situations has been effectively reduced. Could this approach be adopted for other sources or vehicles of contamination?

What are the main sources/vehicles of contamination on a farm? The following is not an exhaustive list, but does outline some areas where control may be possible.

Biological

- Air – not possible to control in fields, but can be controlled in storage facilities by correct positioning of air-intakes and, possibly, filtration.
- Water – already discussed.
- Vegetation – can be controlled by weeding or cutting.
- Soil – it is impractical to attempt to control microbiological contamination of the soil, but the washing of, for example, root vegetables will remove most of the problem.
- Waste products – already discussed.
- Feeding materials – should, if purchased, be accompanied by guarantees with respect to quality and safety.
- Pests – already discussed.
- Domestic animals – not only would it be impossible, but impractical to keep farms free from cats and dogs, for example, although they should be kept in good health and at least farmers should be aware of the potential for contamination from domestic animals. In certain circumstances, such as milking parlours, these animals should be denied access at all times.
- Livestock – as with domestic animals, they should be kept in good health and veterinary advice should be sought whenever necessary.

- Humans – farm workers showing any signs of gastro-intestinal disease should report to the manager and avoid working in areas where direct contamination of food is possible.

Chemical

- Contamination from chemical pollutants – difficult to control and expert advice should be sought about which crops can be grown safely in the circumstances.
- Contamination from naturally occurring chemicals – difficult to control and expert advice should be sought about the most suitable crops.
- Contamination from chemicals that have been introduced – follow the manufacturer's instructions carefully, particularly with regard to appropriate usage, storage, dilution rates, application rates, dose rates and withdrawal times, and consider what, if any, alternative treatments are available.

Physical

- Physical contamination from sticks, stones, insects, small animals, bones, faeces, earth, plant material, metal, and so on – it is, of course, difficult to remove all physical contaminants, but often some form of either mechanical or manual sorting is both viable and necessary.

If all of these factors are taken account of by the farmer, as they should be, then the need to follow a classical HACCP system becomes minimal, except in situations where the production flow lends itself easily to this type of control.

10.9 HACCP in a poultry unit

Narrowing down from general farming and primary production to the main theme of this book, poultry production, can a classical HACCP plan be put in place in a poultry unit? The simple answer is, of course, 'yes', but would it bring any benefit?

As a system, poultry production is no different from any other food manufacturing process. Raw materials are sourced, purchased, accepted at delivery, prepared, processed, packaged and despatched. Flow diagrams and all the other accepted mechanisms of a classical HACCP plan are relatively easy to put in place and implement. However, proper CCPs are few in relation to microbial hazards, so is there any need to go through all the procedures, just to say 'We have a HACCP system in place'? In practice, there is much evidence that *Salmonella*, at least, is controllable on poultry farms, when controlled-environment housing is used (see Chapter 5). The CCPs are, of course, the acceptance at delivery of the day-old chicks and feedstuffs, and the supply of drinking water. If the chicks are free from *Salmonella* on delivery, then good housekeeping, good husbandry, good manufacturing practice, good hygiene practice – call it what you will – will help to keep them free until the day of

despatch. If the feed and water are pathogen-free, then these sources cannot introduce pathogens into the premises or the birds. If, on the other hand, contaminated chicks, feed or water are accepted into the premises, then there is no later CCP on the farm that will either eliminate or reduce to an acceptable level the *Salmonella* contamination.

This does not mean that there is no work to be done after the delivery stage. Biosecurity must be maintained at a high level and aspects such as monitoring of pests and diseases, personal hygiene and post-rearing cleaning and disinfection of the house must be maintained at the highest possible level. It should never be forgotten that the young chicks have little or no immunity or resistance to *Salmonella* infection and the initial production area approaches that of a sterile environment. Therefore, if any pathogen is introduced, a widespread and uncontrollable infection is almost inevitable. Various interventions, such as 'competitive exclusion' treatment, are of course available, but these tend to be unreliable unless supported by the full range of hygiene precautions mentioned above.

In short, acceptance of only pathogen-free chicks, feed and drinking water, together with the use of good housekeeping/husbandry/manufacturing/hygiene practices, will provide a HACCP system for intensive rearing of poultry.

10.10 Future trends

Since 'crystal-ball gazing' is one of the least exact sciences, predicting future trends for HACCP on the farm is rather an ambitious undertaking. Most countries have been rather slow in legislating for food safety controls on the farm compared to, for example, their almost indecent haste to introduce the HACCP system in every other part of the food chain, almost regardless of risk. Many reasons have been suggested for this and a few are listed here, merely to assess whether or not the situation is likely to change in the foreseeable future:

- 'Too difficult to implement on the farm' – as discussed earlier, it could prove too time-consuming and ultimately ineffective to introduce full HACCP controls on the farm, but, although there are many activities to be considered, the process would not be unduly difficult.
- 'Farmers wouldn't understand it' – many people in the mainstream food industry do not really understand the HACCP concept, so what's the difference?
- 'Activities on a farm are too complex' – ever been in a large restaurant kitchen during peak dining hours?
- 'It isn't possible to control hazards and risks on a farm' – if you nod your head here, then you haven't read the rest of this chapter!
- 'Farmers are having a tough enough time without being forced to introduce HACCP' – so are butchers, bakers and everyone else involved in the food industry!

- 'Farmers have a strong political lobby' – as do brewers, distillers, cheese makers, caterers!
- 'Food safety isn't so important on a farm' – that's like saying you only need to worry about mayonnaise when you spread it on a sandwich, just prior to eating it!

That leaves us with only two questions that really need to be answered.

1. Could HACCP be extended to the farm? Quite clearly the answer is 'yes'.
2. Should HACCP be extended to the farm? This is a rather more difficult question and requires a supplementary question – would it be justified by the risks and the benefits?

It would seem that sooner rather than later the legislators will come around to saying that the 'risk' certainly justifies it and the benefits probably do so too.

Already retailers are looking at standards on the farm and there are many voluntary schemes in operation for farmers to show that they have reached certain standards for quality and safety. Pressure will be applied to farmers to meet these standards and, in time, most farmers will be part of these schemes in order to survive financially. History shows that what becomes the industry norm soon becomes the legislative norm – legislation is, after all, merely society's expression of what it deems to be the minimum acceptable standard.

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11

Microbial risk assessment in poultry production and processing

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11.1 Introduction

Colonisation of live poultry and contamination of poultry products with human enteric pathogens, such as *Campylobacter*, and *Salmonella*, are common worldwide. The organisms may be introduced at any point from breeding to preparation of the food in the kitchen and thus control requires consideration of the full production and processing chain (ACMSF, 1996). The Hazard Analysis Critical Control Point (HACCP) system offers the opportunity to minimise colonisation or contamination during production and processing by introducing controls at key stages, monitoring their performance and taking any necessary corrective action, and many producers now follow these principles (DeGraft-Hanson, 2003). In addition, many governments are actively giving food safety guidance to the general public via, for example, the internet and education programmes in schools (see, for example, the Food Standards Agency website in the United Kingdom, www.foodstandards.gov.uk and the Food Safety and Inspection Service website in the United States of America, www.fsis.usda.gov).

The principles of HACCP and government education programmes provide a framework for microbiological risk management within the poultry supply chain. To determine their effectiveness, they can be combined with microbial risk assessment (MRA), a tool that provides predictions of human health risks with and without control measures in place (Kelly *et al.*, 2003). Based on such predictions, governments and producers can set targets and priorities for interventions. Thus, together, HACCP and MRA provide a means of improving public health (WHO website, www.who.int/foodsafety/micro/general/en/).

This chapter gives an overview of MRA and its application in poultry production and processing. It begins with an outline of the general methodology of MRA, including scoping the problem, the key components and the alternative approaches. Examples of poultry MRAs are then presented. These examples focus on *Campylobacter* and *Salmonella*, the main organisms for which MRAs have been developed. Presentation of the examples includes description of the questions being addressed by, and approaches followed in, the individual assessments, as well as a discussion of some of the key differences between assessments for *Salmonella* and *Campylobacter*. Following the presentation of examples, future trends in this area are outlined and useful references given.

11.2 The methodology of MRA

11.2.1 Origins of MRA

Risk assessment has been used for several decades in the nuclear, chemical and finance industries. Its use in food safety is, however, relatively recent and stems from the creation of the World Trade Organisation (WTO) and the Sanitary and Phyto-Sanitary (SPS) agreement. This agreement allows WTO member countries to give priority to food safety over international trade only if a scientific basis for this priority can be demonstrated by means of defendable assessments (Wooldridge *et al*., 1996). The Codex Alimentarius Commission (CAC) sets standards for food in international trade and provides guidance on the conduct of risk assessments to promote coordination between member countries. For microbial risks specifically, the CAC have adopted a framework that consists of four components: hazard identification (HI), exposure assessment (EA), hazard characterisation (HC) and risk characterisation (RC) (CAC, 1999). Together, these components provide a systematic process for evaluating the magnitude of risk and ensuring that predictions are based on current scientific knowledge and understanding (Lammerding and Fazil, 2000).

11.2.2 Hazard identification

HI is the first step in a formal MRA. It provides a description of the hazard, that is, the microorganism and/or toxins produced by the microorganism in the food product of interest, for example, poultry meat or eggs. Normally, the link between the organism in the food product and adverse human health effects has been 'identified' prior to the MRA being undertaken (Lammerding and Fazil, 2000). The first step is concerned, therefore, with outlining the evidence for that link. This will include summarising the principal routes of exposure and the available epidemiological information. Typical information will include a brief description of the microbial genus and relevant sub-types, numbers of reported cases of disease, public health outcomes, including outbreak descriptions and reports of the organism in the food product of concern. Data from surveillance and clinical and experimental sources will be useful for this process.

11.2.3 Exposure assessment

In EA, two quantities are estimated: the frequency (or probability) of exposure to the microorganism associated with the food product and the likely number of organisms ingested. Exposure may occur, for example, via consumption of contaminated food or cross-contamination. To estimate these quantities, an exposure pathway is first constructed. This pathway considers the production and processing stages as a series of modules, each of which will influence the probability of ingestion and numbers ingested. A possible pathway for an MRA concerned with shell eggs and egg products is shown in Fig. 11.1. The pathway commences at the farm and the probability of eggs being contaminated at the time of lay and the number of organisms per egg are estimated. The effects of distribution, storage, processing, preparation and consumption are then considered, and the probability and numbers involved are modified to account for these effects. In each module, the unit may also change, for example, from a whole egg to a food item containing eggs.

The pathway shown in Fig. 11.1 is typical of the so-called farm-to-consumption MRA recommended by the CAC (1999). An equivalent pathway for poultry meat is presented by Kelly *et al.* (2003). To model the changes in frequency of contamination and numbers of organisms involved throughout such a pathway, various types of information are required. These relate to the microorganism, the food product and the consumer. In relation to the organism, it is necessary to have information on the levels in or on the raw material (e.g. in the egg at lay or on the chicken, when leaving the farm for slaughter) and how these may be affected by factors such as farm biosecurity, seasonality, region and producer. Information is also needed on the growth and survival characteristics of the organism in response to environmental factors, such as temperature and pH. Growth and/or inactivation can be accounted for by using predictive microbiology (McMeekin and Ross, 2002; Ross and McMeekin, 2003). This is a quantitative approach that predicts the degree of growth or inactivation in a given period of time under a particular set of environmental conditions. It may be relevant to make such predictions during, for example, the storage and/or preparative stages. Necessary information relating to the food product will cover details of production and processing steps, including times and temperatures, details of sanitation and process controls, methods of packaging, ingredients added to the raw product and the level of mixing or separation of the raw product. Consideration of mixing will be particularly important for products that are further processed, for example, when several eggs are pooled within an egg dish or meat originating from several chickens is combined in a chicken burger. In this case, contaminated units may be mixed with uncontaminated units and thus the frequency of contamination and microbial numbers present will be affected. To account for consumer effects, information on food preparation and cooking practices, frequency of consumption and serving size will be required.

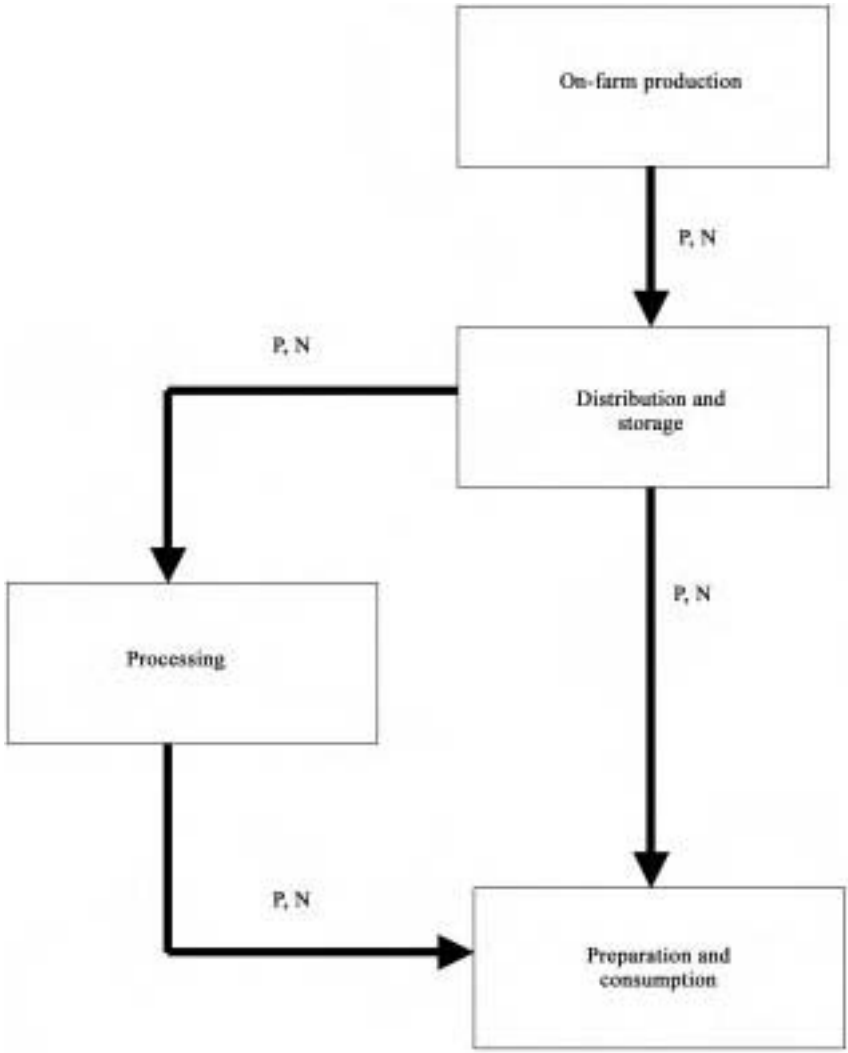

Fig. 11.1 Example exposure pathway for an MRA for shell eggs and egg products (P = probability egg or egg product contaminated, N = number of microorganisms per egg or egg product).

Data for EA

There are many sources of data for EA. These include the published literature and unpublished material, which may be available from, for example, regulatory bodies, food companies or consumer organisations (Lammerding and Fazil, 2000). If the MRA is being undertaken for a national situation, national data will be required for certain parts of the exposure pathway. This will be particularly true when describing effects at the level of the farm and the consumer. The

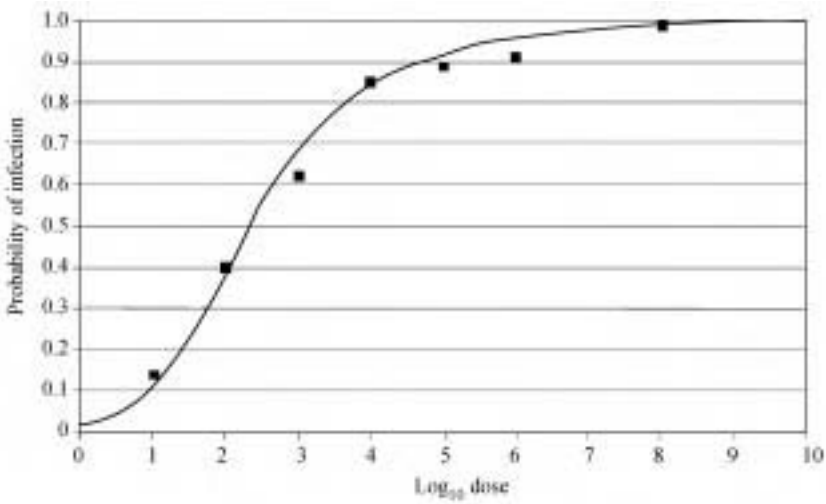

Fig. 11.2 Beta-Poisson dose-response model, fitted to a hypothetical data set.

prevalence of eggs contaminated with a particular microorganism at lay and the frequency of consumption of various egg dishes by different groups in a country will be country-specific and thus require national data. For other parts of the pathway, more general data may be sufficient. For example, when describing inactivation of an organism during cooking of eggs or poultry meat, a well-established growth model could be used for any national situation.

For some parts of the exposure pathway, it may be that there are several data sources and thus corresponding data sets to describe a variable or effect, for example, farm prevalence or temperature during retail storage. When this occurs, the issue of how best to combine the data becomes important. To do so, the assessor must consider the characteristics of the data including, for example, the time-frame over which the data were collected, the sampling scheme and the microbial methods used. These characteristics must be evaluated in relation to the situation being described to determine how representative they are. A weighting may then be used or, alternatively, individual data sets may be rejected.

In contrast to the situation where there is more than one data set available, it may be that there are no or very limited data to describe a variable. This was noted as a particular problem when MRA was first recognised as one of the key factors in managing public health risks from food (Voysey and Brown, 2000). In this situation, expert opinion from, for example, veterinarians, food producers, food scientists, microbiologists and consumer groups can be a valuable source of information. It is important that the opinion is collected in a structured manner that avoids bias. Vose (2000) gives an overview of the important considerations when collecting and combining opinions from several experts.

11.2.4 Hazard characterisation

In HC, the aim is to describe the severity and duration of adverse effects that may result following ingestion of a microorganism or microbial toxin originating from food (CAC, 1999). Ideally, the HC will include a dose-response model, if data are available.

The disease triangle

When undertaking an HC, the risk assessor must take account of factors relating to the microorganism, the host and the food matrix. Together, these three characteristics are known as the disease triangle; they are described in detail by Buchanan *et al.* (2000). In relation to the organism, it is important to have an understanding of the modes of pathogenicity, the virulence factors and how these may change following interaction with the food or the host, the possibility of antibiotic treatment and resistance, the incubation period and persistence time within the host, the potential for person-to-person transmission and the effects at high, medium and low doses. Both individual and population factors must be considered for the host. At the individual level, characteristics such as age, gender, pregnancy, nutritional status and previous medical history will be relevant while, at the population level, access to medical care, population immunity, persistence and secondary transmission may determine the effects of ingestion. Recent research into food-matrix effects has focused on the development of acid resistance in microorganisms and how this may affect their passage through the stomach (Buchanan *et al.*, 2000).

Dose-response modelling

Dose-response modelling is normally the major component of HC. This is a quantitative technique, which involves fitting a mathematical function to data describing the proportions of people exhibiting a response, such as infection or illness (the responses), following ingestion of various numbers of organisms (the doses). Normally, when the proportion of people exhibiting the response is plotted against the logarithm of the number of organisms ingested, a sigmoid or s-shaped curve results. The mathematical functions take a sigmoidal form and are used to predict the response for doses where data are not available. This is known as extrapolation. Often, there are no responses at low doses in the data set and thus low-dose extrapolation is an important consideration when selecting between different dose-response models. An example of a dose-response model for infection with a hypothetical microorganism, fitted to a hypothetical data set, is shown in Fig. 11.2. The function presented here is known as the beta-Poisson model (Haas, 1983), which takes the form given in eqn 11.1:

$$P(\text{Infection} \mid \text{Dose}) = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \qquad 11.1$$

where $P(\text{Infection} \mid \text{Dose})$ is the probability of infection following ingestion of a dose D and α and β are parameters of a beta probability distribution. The beta

distribution describes the variation in the probability of infection amongst hosts and individual organisms. Thus, the model assumes that different individuals will respond differently and different organisms have different abilities to cause infection. If it is assumed that all individuals have the same probability of infection and all organisms have the same ability to cause disease, the simpler exponential model results (eqn 11.2):

$$P(\text{Infection} \mid \text{Dose}) = 1 - e^{-rD} \qquad 11.2$$

where $1 - e^{-rD}$ is the probability of infection following ingestion of a single dose.

The exponential and beta-Poisson models are known as single-hit models. They assume that one organism has the ability to cause infection/illness because of the potential for multiplication within the host. Other single-hit models result if further assumptions, such as clustering of organisms within the food product, are made. These assumptions attempt to reflect biological plausibility and lead to more complex models.

The alternative to the single-hit hypothesis is to assume the existence of a threshold. A threshold is said to exist if no effect is observed below some defined level of exposure, while an effect is certain above that level (FAO/WHO, 2003). This assumption leads to threshold models and these were traditionally used to describe dose-response, particularly for chemical risk assessments.

Data for HC

As with EA, there are potentially numerous data sources for HC and the development of dose-response models. It is important that the risk assessor understands the characteristics of the available data to determine whether or not they can be used as a basis for making meaningful predictions. There are three broad classes of data sources that may be available: human, animal and *in-vitro* studies. The characteristics, strengths and limitations of each are outlined in FAO/WHO (2003).

Human studies include outbreak investigations, feeding trials, surveillance and annual health statistics, biomarker studies and intervention studies. Of these, the feeding trial, in which humans are exposed to different concentrations of the microorganism under controlled conditions, would obviously provide the 'best' data. However, there are severe ethical and economic constraints associated with this type of study, so that few have been carried out. Where such studies have been made, they usually involve only a sub-set of the population and one not normally considered 'at-risk'. The *Campylobacter* feeding trial data published by Black *et al.* (1988), for example, is based on only a group of healthy males. To overcome the constraints associated with human feeding trials, animal studies can be used. However, this approach is limited by the fact that the results must be correlated with those expected for humans, and often there are few data to describe such a correlation.

In cases of severe data limitations, it may be possible again to use expert opinion. As discussed for EA, it is important that standard methods are used to collect and combine such data.

11.2.5 Risk characterisation

RC involves integrating the results from the EA and HC. The outputs from the EA (probability and level of exposure) are fed into the HC to provide estimates of risk, such as the risk of infection per serving or cross-contamination event, or the annual number of cases.

In both the EA and HC, there will be variation and uncertainty associated with the data inputs. These characteristics will be discussed more fully later in this chapter. The estimates of risk must include a measure or description of the associated uncertainty and variability. This may be in the form of a confidence interval, for example. It is also important that the steps of the EA and HC which contribute the most uncertainty and/or variability to the risk are identified. The identification of uncertain steps allows data needs to be communicated and future research to be prioritised. The identification of variable steps enables risk managers to investigate and thus establish appropriate control programmes. This involves changing an assumption or variable, for example, the prevalence of contaminated chickens at slaughter, the addition of a decontamination step during processing or the storage time in the home of the consumer, and re-making the assessment to determine the modified estimates of risk. The options investigated can then be ranked according to the estimates of risk, as well as factors relating to feasibility and cost.

The key assumption underlying any MRA is that the microorganism will have an adverse human health effect on the population. Another important element of RC is the evaluation of the severity of this adverse effect (Buchanan *et al.*, 2000). To do this, different biological end-points, for example, infection, illness or death, can be considered in the HC and used in, for example, a cost-benefit analysis.

Although RC involves integrating the results from the EA and HC, each of these components can also be used as stand-alone products (Kelly *et al.*, 2003). The EA may be useful for producers and processors concerned with reducing the levels of organisms on their finished products. In addition, the HC could be used in MRAs for other food products.

11.2.6 Scoping the problem

Before the MRA is undertaken, it is important that sufficient consideration is given to defining or scoping the problem. This will depend on the risk management question, which will be defined by the underlying reason for undertaking the assessment, for example, to reduce the number of cases of human infection or to facilitate international trade. Background information should be collected from regulators, scientists, food producers and consumer groups, and assembled by the risk manager to give a 'risk-profile' (Lammerding and Fazil, 2000). This

allows definition of the estimate of risk to be calculated, for example, the risk per serving and/or the annual number of cases.

Consideration of problem scope and the risk management question will also define the scope of the assessment. In particular, it may not be necessary to develop a full farm-to-consumption assessment (Fig. 11.1), and a simpler approach may suffice (Kelly *et al.*, 2003).

11.2.7 Types of MRA

There are two main types of MRA: qualitative and quantitative. Both should follow the same systematic process (Lammerding and Fazil, 2000); they differ in the amount and type of data required.

Qualitative MRA

Qualitative assessments involve assigning categorical descriptions to the variables and steps on the exposure pathway and in the RC. Terms such as negligible, low, medium or high may be used to do this. Assigning these terms requires a logical consideration of the available information. For example, information relating to prevalence, biosecurity, vaccination, seasonality and producer would be used to categorise the probability of eggs being contaminated with a particular pathogen at the time of lay, the first step on the exposure pathway shown in Fig. 11.1.

Undertaking a qualitative evaluation should be the first step of the MRA, and the outcome will determine whether or not it is feasible or necessary to develop a quantitative model. If the qualitative evaluation suggests that the risk is negligible or very low, for example, it may not be considered worthwhile to invest extra resources in developing a quantitative model. Similarly, if the qualitative evaluation highlights several important data gaps, it may be concluded that it is not possible to develop a fully quantitative model. It may be, for example, that a quantitative model commencing at a stage beyond the farm may be the only feasible quantitative approach.

Assigning and combining the categorical terms within a qualitative MRA is probably the most difficult exercise, because assessor bias may arise. In particular, there may be disagreement on what is meant by low or high risk and on how these values should be combined at, for example, different stages of the exposure pathway. To overcome this problem, it is crucial that the assessment is transparent and that all evaluated information is made available. This allows informed debate on what constitutes a particular level of risk. To aid transparency, any system used to define and combine levels should be documented. See Moutou *et al.* (2001) for an example of a qualitative assessment that includes transparent documentation.

Quantitative MRA

In quantitative MRA, numerical estimates of risk are produced. This requires numerical data for each step on the exposure pathway and to define a dose-

response model. The data are used within a mathematical model that describes the relationship between the various inputs and how they combine to give the estimate of risk.

There are two approaches to developing a quantitative MRA: point-value or probabilistic. In a point-value assessment, each input is described by a single value, such as the mean, the most probable or the worst-case. This results in a point-value for the estimated level of risk. The problem with the point-value approach is that inherent variation (variability) and/or uncertainty (lack of information) are not included. These characteristics are very likely to be associated with the data collected. There may be variation between, for example, farms, seasons, producers, processing plants and consumers, while uncertainty may arise because of, for example, small sample sizes, a lack of detail concerning processing steps and a lack of available data. Ignoring these effects will lead to a loss of information (Lammerding and Fazil, 2000).

To account for variability and uncertainty, the probabilistic assessment can be used. Here, probability distributions are used to describe model inputs. These distributions describe the range of possible values that an input can take and the relative frequency or probability with which any given value will occur. Thus, they account for extreme values, for example, the highest or lowest concentrations of organisms, as well as those that occur 'on average'. Probability distributions are characterised by a number of parameters, for example, the mean and standard deviation. These parameters are derived from numerical data that may come from any of the previously described sources. The distributions can describe uncertainty and/or variability; however, it is important that these characteristics are kept separate in order that the estimated levels of risk can be described in terms of both uncertainty and variability. This enables the risk manager to investigate and/or deal with them appropriately.

Once the probability distributions have been defined, they are used within the same mathematical model as that used in the deterministic case; the point-values are simply replaced by the distributions. However, the distributions add to the complexity of the model in that the risk distribution cannot be derived theoretically. To overcome this problem, a computer-based numerical method known as Monte-Carlo simulation is often used. In this method, values from each probability distribution are randomly sampled according to the frequency with which they occur. The random values are then combined according to the mathematical model and the result stored. This process is repeated many times (iterations) and all stored values are combined to define the probability distribution of risk. The process is described schematically in Fig. 11.3 for a simple model, which estimates the log number of microorganisms per egg at the point of preparation, based on the log number of organisms at lay and potential growth during distribution and storage. It is assumed that the number of organisms per egg at lay is variable and described by a distribution that can range from 0 to 3. Similarly, the log amount of growth during distribution and storage will be variable, depending on, for example, temperature and time, and is assumed to range between 0 and 2. On each iteration, a value is randomly

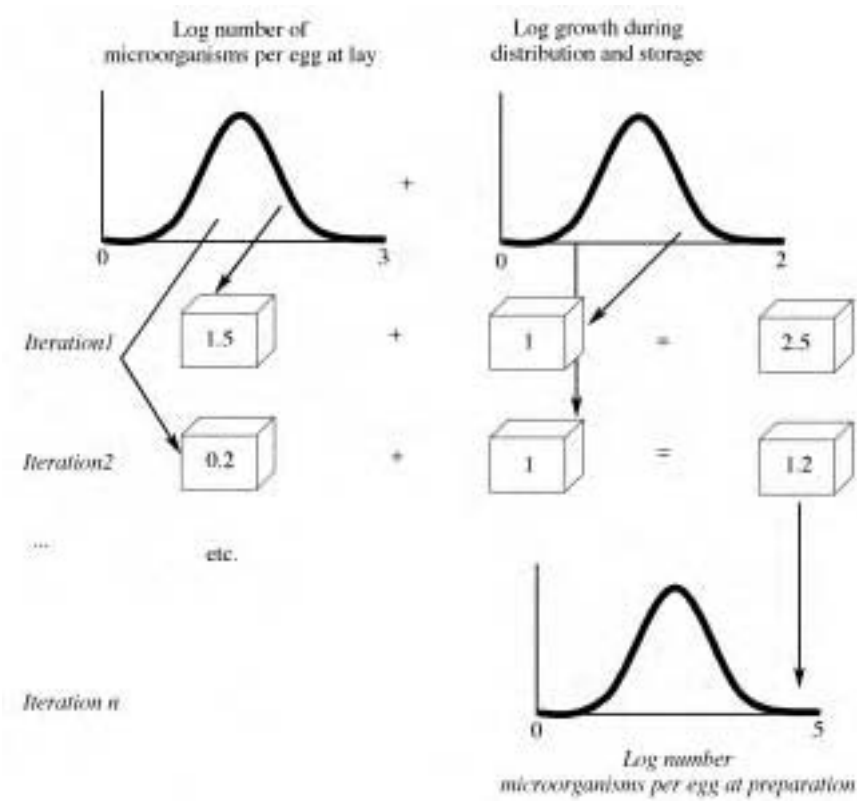

Fig. 11.3 Schematic representation of a Monte-Carlo simulation that estimates the log number of microorganisms per egg at preparation and consumption, based on the log number at lay and potential growth during distribution and storage. The parameter *n* is the number of iterations.

generated from each distribution and added together to give the log number of microorganisms at the point of preparation. All *n* values define the distribution.

The process of generating random values from probability distributions is based on complex statistical theory. This theory is encompassed in computer software specially designed for undertaking Monte-Carlo simulation, for example, the package @RISK (© Palisade Corp.). Alternatively, statistical packages or programming languages can be used to code the models.

11.2.8 Validation of MRAs

Validation of MRAs, particularly quantitative assessments, is a highly debated issue. Although it would be very desirable to validate predictions with observations, the process is usually fraught with difficulties. These occur because of, for example, under-reporting of cases of illness and simplifying assumptions made within the assessment (Kelly *et al.*, 2003). The difficulties can be overcome to

some extent by, for example, validating outputs at earlier stages of the exposure pathway. In addition, the assessment can be used in a relative sense, for example, by comparing estimates of risk under different scenarios. It is also important to remember that the key outputs of an MRA are the identification of data gaps and control strategies, rather than the actual estimates of risk.

11.3 MRAs for *Salmonella* and *Campylobacter*

To date, the vast majority of poultry MRAs or part-MRAs have been developed for *Salmonella*, *Campylobacter* and antibiotic-resistant organisms. Table 11.1 lists a collection of those currently available in the literature. For each assessment, a summary of the key features is provided alongside details of the microorganism and poultry product concerned. It is likely that other MRAs exist, but have not yet been published. Thus, the list offers selected examples.

Most of the assessments in Table 11.1 are quantitative. Most focus on a variety of products and thus consider several exposure pathways. All of the models for *Campylobacter* in broilers consider the full farm-to-consumption pathway, whereas those for *Salmonella* in broilers commence later in the production chain or consider only part of the exposure pathway. This is a reflection of the differences in available data for the two organisms. For *Campylobacter*, data are available to quantify the changes in numbers during processing, whereas, for *Salmonella*, such comprehensive data sets are not available (FAO/WHO, 2002). On the whole, the *Campylobacter* models are based on the same data sets to describe the effects of broiler processing, although they incorporate different statistical methods. For example, the model of Hartnett (2001) explicitly separates uncertainty and variability, while those by Fazil *et al.* (1999) and Rosenquist *et al.* (2003) do not. For *Salmonella* in eggs, the most recent models consider the full farm-to-consumption pathway; again, this is a reflection of data availability.

Another difference between the models for these two organisms is that those for *Salmonella* tend to take account of microbial growth, whereas those for *Campylobacter* do not. This is a reflection of the growth characteristics of the organisms in that the production chain is unlikely to include temperatures high enough for *Campylobacter* to grow, although this may not be the case for *Salmonella*. The FAO/WHO (2002) model for *Salmonella* in broilers, for example, assumes that growth could occur during storage at retail, during transport or in the home, if the temperature exceeds 10 °C. This growth is predicted by a model presented by Oscar (1999). In the models for *S.* Enteriditis in eggs, growth is assumed to depend on the permeability of the yolk membrane, which changes over time and in response to temperature. Once the breakdown of the membrane is complete, the organisms are then assumed to grow according to a predictive model (see FAO/WHO (2002) for a review on the different growth models used).

Many of the models listed in Table 11.1 estimate human health risks for a national situation. Thus, they use national data, where appropriate, to describe, for example, prevalence and consumption patterns. The FAO/WHO models

Table 11.1 Published MRAs for *Salmonella* and *Campylobacter* is poultry

Microorganism	Poultry product	Key features	Reference
Salmonella *Enteriditis*	Eggs	• USA • Mainly qualitative • Summary of outbreaks and risk factors	Morris (1990)
Salmonella	Cracked eggs	• Canada • Mainly quantitative • Analysis of data on reported cases and numbers of cracked eggs to derive a relative risk	Todd (1996)
Salmonella *Enteriditis*	Pasteurised liquid egg	• USA • Quantitative • Farm-to-consumption model focusing primarily on mayonnaise made with pasteurised liquid egg	Whiting and Buchanan (1997)
Salmonella *Enteriditis*	Shell eggs	• USA • Quantitative • Farm-to-consumption model focusing on an exhaustive list of consumption pathways	USDA-FSIS (1998)
Salmonella *Enteriditis*	Eggs	• International • Quantitative • Review of available models with some additional modelling and investigation of mitigation strategies	FAO/WHO (2002)
Salmonella	Broiler chickens	• USA • Quantitative • Demonstration model focusing on processing	Oscar (1997)
Salmonella	Broiler chickens	• USA • Quantitative • Demonstration model focusing on processing, preparation and consumption	Oscar (1998)

Table 11.1 (continued)

Micro-organism	Poultry product	Key features	Reference
Salmonella	Chicken products	• Quantitative • Model incorporating prevalence and effects of cooking	Brown *et al.* (1998)
Salmonella	Broiler chickens	• International • Quantitative • Processing-to-consumption model	FAO/WHO (2002)
Campylobacter jejuni	Broiler chickens	• Canada • Quantitative • Farm-to-consumption model focusing on exposure via cross-contamination and undercooked products	Fazil *et al*. (1999)
Fluoroquinolone-resistant *Campylobacter jejuni*	Broiler chickens	• USA • Quantitative • Assesses relationship between prevalence in chicken products at retail and human cases	FDA/CVM (2000)
Campylobacter jejuni	Broiler chickens	• UK • Quantitative • Farm-to-consumption model focusing on exposure via cross-contamination and undercooked products	Hartnett (2001)
Campylobacter jejuni	Broiler chickens	• International • Quantitative • Review and integration of available farm-to-consumption models	FAO/WHO (2001)
Campylobacter jejuni	Broiler chickens	• Denmark • Quantitative • Farm-to-consumption model focusing on cross-contamination	Rosenquist *et al*. (2003)

(FAO/WHO, 2001, 2002) have attempted to aggregate the features of some of these national models to provide a framework that can be used in countries where resources are not available to produce large, quantitative models, such as that of the USDA-FSIS (1998) for *S.* Enteriditis. The FAO/WHO model for *Campylobacter* integrates some of the features of the models of Fazil *et al.* (1999), Hartnett (2001) and Rosenquist *et al.* (2003).

11.4 Future trends

The use of MRA for managing food safety risks has seen a significant expansion in recent years. This is evident from the number of large-scale quantitative models that have been produced and used for decision-making. Table 11.1 provides examples of poultry models, and similar advances have been made for other microorganisms and food products (see, for example, Cassin *et al.*, 1998 and Nauta *et al.*, 2001). In the future, it is likely that the trend will continue, with more and more countries using this tool both to manage national risks and for the purposes of international trade. The available model frameworks and guideline documents (for example, FAO/WHO, 2002) are resources that will aid this development.

The identification of data gaps should be one of the key outputs from MRA. Almost all the publications outlined in Table 11.1 indicate areas of the model that could be improved, if additional data were available. The FAO/WHO models for *Salmonella*, for example, list data requirements, such as numbers of organisms at different stages of production and processing, frequency of consumption and serving sizes and preparation patterns both in the home and in catering establishments (FAO/WHO, 2002). Risk managers and microbiologists are often surprised by claims of 'limited data', as they are aware of extensive microbial studies. However, these studies often do not generate data that fits easily into MRAs. For example, to model the full farm-to-consumption chain for broilers, studies are required that provide the numbers of organisms per bird or carcass at each stage of processing while, in reality, only presence/absence data are usually available. This is not surprising, because the studies are unlikely to have been designed to provide data for MRA. To overcome this problem, it is necessary that MRA is used to drive the process of data generation. This can be achieved by undertaking MRAs and experimental studies in parallel. Here a preliminary model would be set up and then exact requirements communicated to those designing the experimental studies. This is beginning to happen already (see Stern *et al.*, 2003 and Snary *et al.*, 2003, for example) and is likely to continue to a much greater extent in the future.

11.5 Sources of further information and advice

As the importance of MRA in managing public health risks has become more widely recognised by risk managers, food producers and international bodies, the

available guidance material has grown at a rapid pace. For a general overview of the process, the CAC guidelines (CAC, 1999) provide a useful starting point. These guidelines present the Codex framework discussed previously, as well as general terminologies and definitions. The WHO and the FAO are currently involved in a programme of MRA activities for the Codex Commission on Food Hygiene and member countries. The programme includes a series of Joint FAO/WHO Expert Meetings on Microbial Risk Assessment, the objectives of which include the provision of risk assessments for specific pathogen and commodity combinations, and guideline documents. Full details of the meetings, risk assessments and documents produced can be found at the FAO and WHO websites (www.fao.org/es/ESN/food/risk_mra_riskassessment_en.stm and www.who.int/foodsafety/micro/en/).

The International Life Sciences Institute is also heavily involved in the production of guidelines and arrangement of activities on MRA. A guidance document has been published that outlines the principles of risk assessment, including MRA, and a session on MRA was held at Food Micro '99, the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene. The proceedings of this session are published in the *International Journal of Food Microbiology* (Volume 58, Issue 3, 2000) and give an overview of the process. The papers by Buchanan *et al.* (2000) and Lammerding and Fazil (2000) are particularly useful for HC and EA, respectively. Other useful general documents include Coleman and Marks (1999) and Kelly *et al.* (2003).

Many of the MRAs outlined in Table 11.1 are available at the Food Safety Risk Analysis Clearinghouse website (www.foodriskclearinghouse.umd.edu). The Clearinghouse collates and catalogues data and methodological information on food safety risk analysis from international sources. It thus provides a helpful introductory source of information, as well as a source of data for practitioners.

For those readers interested in actively pursuing quantitative MRA, useful textbooks include Cullen and Frey (1999), Haas *et al.* (1999) and Vose (2000).

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12

Techniques for reducing pathogens in eggs

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12.1 Introduction

Foods of animal origin, including dairy products, meat, poultry and eggs, have long been recognized as the primary source of many of the bacteria responsible for foodborne infections and intoxications (Roberts, 1990; Jay, 1992b). Raw foods of this kind are especially susceptible to contamination by bacterial pathogens, which may be present within or on healthy animals used in food production. Many such pathogens are also ubiquitous, and sources of contamination for raw or processed egg products include: food-production animals, soil, water, equipment surfaces and food handlers.

The public health significance of even relatively small populations of pathogens in foods at the time of consumption is an issue of growing concern and public policy debate. An increasing proportion of the US and world populations (including the aged, organ transplant recipients and the immunodeficient) is becoming especially susceptible to a wide variety of pathogens. In addition, various bacteria are either being newly recognized as causes of human enteritis, or it has been shown that illnesses, originally thought to spread via person-to-person or animal-to-animal routes, are transmissible by the ingestion of contaminated foods (Madden, 1994).

As a food of animal origin, eggs may be contaminated with a variety of potentially pathogenic microorganisms. Based on the published literature, a list of currently-recognized bacterial pathogens with a reasonable likelihood of occurring in blended, raw-egg products could include: *Salmonella enterica* ss. *enterica*, *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Aeromonas hydrophila, Yersinia enterocolitica* and *Campylobacter* spp. The

microbiological safety of raw-egg products, contaminated with a given pathogen, will depend ultimately on:

- the in-going population of pathogenic microorganisms
- the adequacy of the thermal process used (e.g. pasteurization, ultra-pasteurization), or other pathogen-reduction treatment
- the cooling rate
- the hygienic quality of the filling operation in the case of liquid egg products
- the time-temperature profile during distribution and storage
- the degree to which proper food-handling techniques are used at the point of consumption.

Of the above-listed pathogens, *Salmonella* serotypes and, in particular, serotype Enteritidis, have the greatest historical association with contaminated eggs and egg products. In addition, *L. monocytogenes* and *A. hydrophila* pose particular concerns, if present in chilled, liquid egg products, due to their ability to multiply in foods at proper, refrigerated storage temperatures (e.g. 5 ºC). However, there have yet to be any documented cases of illness involving these two organisms that could be linked to the consumption of eggs or egg products.

Statistics indicate that up to 90% of US foodborne disease outbreaks are attributable to improper handling, cooking and storage practices in food-service operations or in the home (Bean and Griffin, 1990). Nonetheless, when food processed by a large, high-volume manufacturer is involved, the potential for a large-scale outbreak increases proportionately (Bean and Griffin, 1990; CAST, 1994). Moreover, the negative publicity incurred, when a large company is implicated in a foodborne disease outbreak, may be devastating to the company's image within the marketplace.

A key aspect of food safety programs is the identification of factors that can be manipulated to inactivate or control the microbial pathogens that are most likely to jeopardize the safety of finished products. Examples of such factors are the formulation of the food product (and especially the presence of preservatives or 'hurdles' to microbial growth), the severity of the thermal process (e.g. pasteurization), or other pathogen-reduction treatment, and the sanitary condition of the packaging operation. In recent years, advances in egg processing technologies have permitted production of heat-processed, liquid egg products with a shelf-life of 8–10 weeks at 4 ºC. Because many such products provide nearly ideal growth conditions for a wide variety of bacterial pathogens, tolerances for viable micro-organisms in finished, processed (i.e. pasteurized) products are relatively small.

In this chapter, a summary is given of the US Department of Agriculture (USDA) risk assessment for *Salmonella* in shell eggs and egg products, a foodborne illness report involving eggs from the US Centers for Disease Control and Prevention (CDC), a description of the pending US government egg-safety initiative, a section on rapid egg cooling, comprehensive coverage of thermal and non-thermal egg pasteurization technologies, sections on the thermal resistance of *Salmonella* serotypes, *Listeria monocytogenes* and *Aeromonas hydrophila* in eggs and egg products and a concluding section on future trends in egg safety.

12.2 USDA *Salmonella* Enteritidis (SE) risk assessment for shell eggs and egg products

The risk assessment report, published on June 10, 1998 by the USDA Food Safety and Inspection Service (FSIS) summarized the risk assessment process from the development of a conceptual framework to the careful organization of information obtained from published scientific literature and unpublished academic, government and industry sources. The available data was incorporated into a comprehensive, quantitative model that characterized the public-health effects associated with the consumption of SE-infected shell eggs and egg products (USDA, 1998).

The FSIS began its risk assessment in December 1996 in response to an increasing number of cases of human salmonellosis associated with the consumption of shell eggs. The objectives of this risk assessment were to determine the unmitigated risk of foodborne illness from SE, identify and evaluate potential risk-reduction strategies, establish data needs and prioritize future data-collection efforts. The risk assessment model consisted of five modules. The first one, the Egg Production Module, estimated the number of eggs produced that are infected (or internally contaminated) with SE. The Shell Egg Module, the Egg Products Module and the Preparation and Consumption Module estimated the increase or decrease in numbers of SE in eggs or egg products as they passed through storage, transportation, processing and preparation. The Public Health Module then calculated the incidence of illness and four clinical outcomes (recovery without treatment, recovery after treatment by a physician, hospitalization and mortality), as well as the cases of reactive arthritis associated with consuming SE-positive eggs.

The baseline model for shell eggs presented in this report assumed an average production of 46.8 billion shell eggs (of the total 65 billion eggs produced) per year in the USA, 2.3 million of which were projected to contain SE (0.0049% infectivity rate or about 1 in 20,000 eggs being *Salmonella*-positive). The consumption of these contaminated eggs resulted in a mean estimate of 661,633 human cases per year, ranging from 126,374 to 1.7 million cases (5th and 95th percentiles), as shown in Table 12.1. It was estimated that about 94% of these cases would recover without medical care, 5% would visit a physician, an additional 0.5% would be hospitalized and 0.05% of the cases would result in death. Twenty percent of the population was considered to be at a higher risk of salmonellosis from SE (i.e. infants, the elderly, transplant patients, pregnant women, individuals with certain diseases), because those concerned may be more susceptible to infection and experience disproportionately the manifestations of SE infection.

A comparison of the total number of cases due to SE-positive eggs that were associated with this model and the distribution of illness from such eggs that was predicted from national, public-health surveillance data, shows a substantial overlap between the two independently-derived distributions (see Fig. 12.1). The surveillance data was used to obtain an estimate of SE-related human illness

Table 12.1 Public health outcome summary (*Salmonella* Enteritidis risk assessment of shell eggs and egg products)*

Category	5th percentile	Mean	95th percentile
Exposed	536,583	2,410,904	5,836,237
Ill	126,374	661,633	1,742,592
Recover w/no treatment	118,806	621,684	1,626,680
Physician visit and recovery	7,235	36,208	93,259
Hospitalized and recovered	627	3,350	9,382
Death	68	391	1,050
Reactive arthritis	3,631	19,994	55,915

* USDA (1998).

linked to eggs that averaged 637,000 cases per year, with a range from 254,000 to 1,167,000 cases. The median estimates for the model and the surveillance data were 504,082 cases and 332,400 cases per year, respectively. The closeness of the two values suggests that the model is reasonably accurate in its prediction of the number of SE cases in the USA that were due to SE-positive eggs. This model deals only with SE cases caused by the consumption of eggs that were contaminated internally (i.e. vertical route of contamination) and does not account for other possible sources of human illness due to SE.

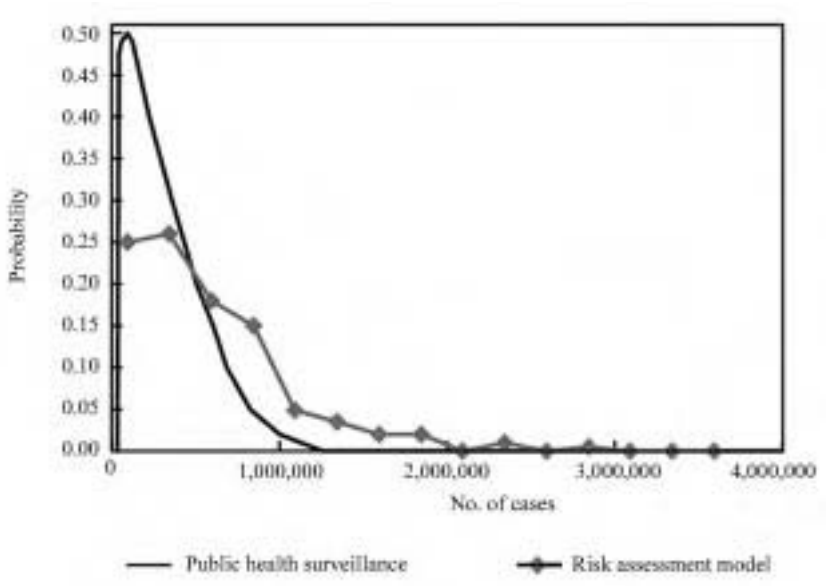

Fig. 12.1 Annual human *Salmonella* Enteritidis cases from eggs – comparison of data from public health surveillance with that predicted by the risk assessment model (USDA, 1998).

The baseline egg-products model predicted a low probability that any cases of salmonellosis from SE would result from the consumption of pasteurized egg products. However, the FSIS pasteurization regulations do not provide sufficient guidance to the industry for the large range of products currently being produced. Time and temperature requirements, based on the numbers of bacteria occurring in the raw product, the method of processing the raw material and the intended use of the final product need to be taken into account to provide greater protection for consumers.

A proportionate reduction in human salmonellosis was calculated for two scenarios within the Shell Egg Processing and Distribution risk module. In the first scenario, a 12% reduction in illness was projected, if all eggs were cooled immediately after lay to an internal temperature of 7.2 ºC and then maintained at that temperature throughout processing and distribution. This was in contrast to the diversity of temperatures actually experienced. With regard to the effect of storage temperature, the USDA has now amended the regulations and requires that shell eggs intended for human consumption be stored and transported under refrigeration at a temperature of 7.2 ºC. In the second scenario, it was found that an 8% reduction in illness would occur when eggs were maintained at an environmental temperature of 7.2 ºC throughout processing and distribution. These two scenarios represented the best results that could be expected from implementing temperature control at the stages in question.

Mitigation elasticity measures the effects of specific interventions. Thus, the effect was determined of diverting eggs from SE-positive flocks out of the shell-egg market and into the egg-products sector for pasteurization. There was a substantial reduction in the number of human cases arising from 25% fewer SE-positive eggs being available to shell-egg consumers.

12.3 CDC foodborne illness report

A SE epidemic emerged in the 1980s, when increasing numbers of infections were detected in the Northeastern and Mid-Atlantic regions of the USA (Mishu *et al*., 1994). In the early 1990s, SE rates in the Northeast began to decline, but the SE epidemic extended to the Pacific region (Angulo and Swerdlow, 1998). Nationwide, the number of SE isolations reported to CDC peaked at 3.8 per 100,000 of the population in 1995. Although rates of culture-confirmed SE infection reported to CDC had declined to 1.9 by 1999 (Fig. 12.2), rates did not decline further through 2001. Investigations of outbreaks and sporadic cases have indicated repeatedly that, when a food vehicle is identified, the most common sources of SE infection are undercooked and raw shell eggs (St Louis *et al*., 1988; Angulo and Swerdlow, 1999). The CDC report described two SE outbreaks associated with eating shell eggs and emphasized the need to strengthen existing SE control measures.

During 1990–2001, state and territorial health departments reported 677 SE outbreaks, which accounted for 23,366 cases, 1,988 hospitalizations and 33

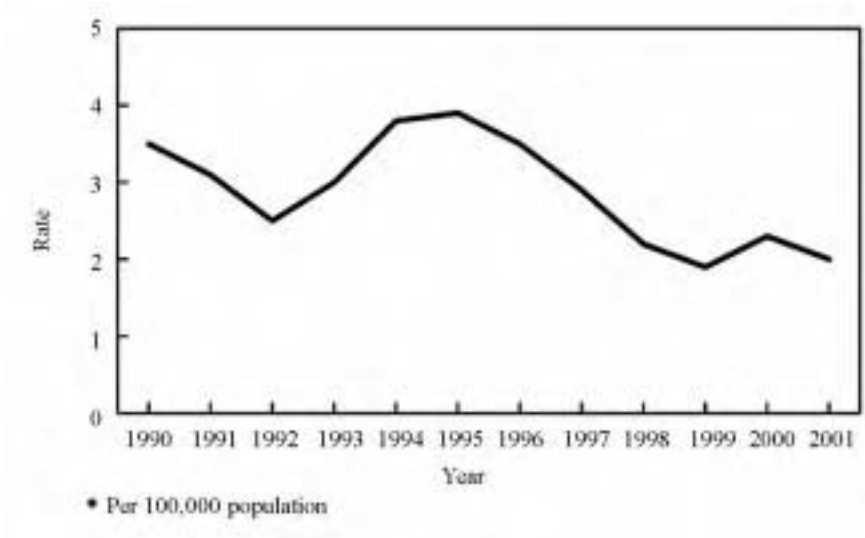

Fig. 12.2 Isolation rate* of *Salmonella* serotype Enteritidis (SE) by year – United States, 1990–2001 (Centers for Disease Control and Prevention, 2003).

deaths (CDC, 2002, unpublished data). Among the 309 reported outbreaks with a confirmed vehicle of transmission, 241 (78.0%) were associated with shell eggs, accounting for 14,319 cases. Of these, 10,406 cases occurred during 1990–1995 and 3,913 during 1996–2001. The overall decrease in SE incidence and the decrease in the number of cases related to egg-associated SE outbreaks during the last decade could be attributed in part to the implementation of preventative measures, including on-farm control programs, egg refrigeration and consumer and food-worker education. However, the number of reported cases did not decline during 1999–2001, and outbreaks associated with shell eggs continue to occur.

To achieve sustained decreases in egg-associated SE illnesses, a concerted preventative effort is needed, from the farmer to the consumer (President's Council on Food Safety, 1999). A key factor in this effort is the implementation of farm-based measures to reduce SE contamination of eggs during production. The implementation of such control programs in the Northeastern United States in the early 1990s could have contributed to subsequent decreases in human cases in the New England and Mid-Atlantic regions (Hogue *et al.*, 1997). An important part of the control measures was the microbiological testing of hen houses for the presence of SE. If SE is found on a farm during routine environmental testing, eggs may be diverted to pasteurization. Evidence suggests that proper implementation and supervision of farm-based control programs can result in a reduction of SE infections among housed poultry flocks (White *et al.*, 1997). Farm participation in current SE control programs is voluntary, and the components of different programs can vary. Future shell-egg

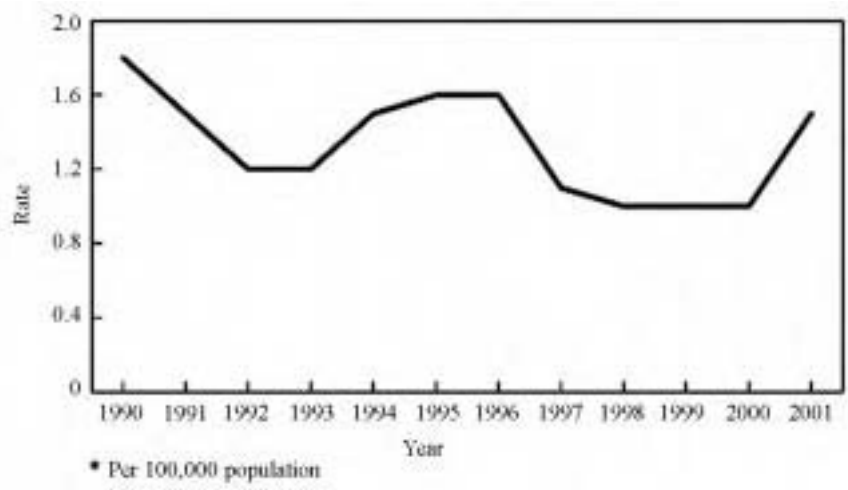

Fig. 12.3 Isolation rate* of *Salmonella* serotype Enteritidis (SE) by year – Southeastern Region, 1990–2001 (Centers for Disease Control and Prevention, 2003).

safety measures should include greater participation in farm-based programs, with microbiological testing.

Both outbreaks described in this report occurred in the Southeastern region of the USA. Compared with the declining rates of SE infection in other regions of the country, the incidence of SE in the Southeastern states increased by 50% from 2000 to 2001 (Fig. 12.3). Ongoing surveillance of SE outbreaks will be necessary to detect any changing trends. Expansion of control measures will be an important part of efforts to prevent SE infection in the Southeast. This includes actively encouraging farms to participate in SE control programs, promotion of proper refrigerated transport and storage of eggs and education of food handlers and consumers about food preparation. Retailers and consumers can specify that suppliers provide only eggs produced on farms with an SE control program that is recognized by a state regulatory agency or a state poultry association.

Because individuals that are confined to institutions depend entirely on these establishments for their meals, any use of contaminated foods can place large populations at risk of developing foodborne disease. The residents, especially elderly people in nursing homes or assisted-living facilities, are particularly prone to dying from outbreak-associated SE infections (Levine *et al.*, 1991). During 1990–2001, a total of 83 SE outbreaks occurred in institutional settings, representing 12% of all reported SE outbreaks. Of the 33 outbreak-associated deaths, 22 (67%) occurred in institutions, indicating the importance of using pasteurized egg products or in-shell pasteurized eggs for all recipes requiring pooled, raw or undercooked shell eggs for people in this type of situation.

12.4 Egg safety from production to consumption: an action plan to eliminate salmonellosis due to SE contamination of eggs

Americans consume an average of 234 eggs per person per year. Although eggs are an important source of protein in the diet, it has been estimated that 1 in 20,000 eggs in the US supply will contain SE and can cause illness, if used raw in foods or only lightly cooked before consumption. Because eggs can become contaminated internally during formation in the hen, certain egg-handling practices are now considered to be unsafe. These practices include the keeping of eggs and egg-containing foods at room temperature, instead of under refrigeration, inadequate cooking and pooling eggs to prepare a large amount of an egg-containing food that is subsequently temperature-abused or inadequately cooked.

The SE risk assessment model for shell eggs and egg products described above was developed jointly by the FSIS and the US Food and Drug Administration (FDA) in 1998. It predicted that simultaneous use of multiple interventions could achieve a more substantial reduction in SE-related illness than any one intervention alone. This finding suggests that a broadly-based control policy is likely to be more effective than a policy directed at only one stage of the egg production-to-consumption continuum.

On August 26, 1999, the US President's Council held a public meeting to canvass opinion for the development of an action plan to address egg safety (President's Council on Food Safety, 1999). A single theme resounded from representatives of both consumer groups and the egg industry: the federal government needed to set mandatory national standards. These standards should:

1. provide consumers with an assurance that all eggs are subject to the same safety standards across the US
2. provide a 'level playing field', industry-wide, for egg producers and processors.

At each stage of the egg production-to-consumption continuum, the preliminary plan identifies the systems and practices necessary to reduce and, ultimately, eliminate eggs as a source of human SE infection. The plan offers industry the flexibility to choose from two, equivalent SE-reduction strategies, each delivering eggs into the distribution chain and to the consumer at a comparable level of safety. The overall goal of the Council was to eliminate egg-associated illness from SE by 2010. However, the interim goal of the Egg Safety Action Plan was a 50% reduction in illness by 2005. Since the final rule has yet to be announced, these goals may be adjusted accordingly. The strategy chosen by egg producers and packers/processors determines the point at which the pathogen-reduction steps are taken:

- Strategy I: SE testing and egg-diversion system on farm
- Strategy II: Lethal treatment, or 'kill step' at packer/processor establishment.

For the distribution and retail stages, the plan specifies the handling practices necessary to ensure that consumers receive a safe food product. Furthermore, the

plan describes clearly the surveillance, research and educational activities that must also be conducted to achieve the elimination of egg-associated SE illness. A comparative summary of the activities in Strategies I and II is provided in Table 12.2.

To consolidate the supervisory responsibilities involved and provide clarity, the President's Council on Food Safety identified one responsible agency for each stage of the farm-to-table continuum, based on the strengths of each one, as follows:

- FDA develops standards for the producer and individual states provide inspection and enforcement on the farm.

Table 12.2 Egg safety action plan; comparative summary of strategies I and II

Comparison of Program Strategies for Action Plan (President's Council on Food Safety, 1999)

Activity	Strategy I	Strategy II
Production (On-Farm): Objective 1.1		
Chicks from SE-free breeder flocks	X	X
SE Environmental Testing (chicks, pullets, layers)	X	
SE Environmental Testing (at depopulation)	X	X
SE Egg Testing (w/positive environmental results)	X	
Diversion of shell eggs to pasteurization	X	
Biosecurity	X	X
Rodent/Pest control	X	X
Decontamination (Cleaning & Disinfection)	X	X
Packing[1]: Objective 1.2		
Prerequisite programs	X	X
HACCP system with a 'kill' step		X
Processing[1]: Objective 1.3		
Prerequisite programs	X	X
HACCP system with a 'kill' step	X	X
Distribution[2]: Objective 1.4		
Refrigeration during transport and storage	X	X
Retail: Objective 2		
Food Code (egg-relevant provisions)	X	X
Surveillance: Objectives 3–6		
Monitoring human and poultry SE infections	X	X
Research: Objective 7	X	X
Education: Objective 8	X	X

[1] Prerequisite programs must address: basic sanitation of facilities and premises; rodent and pest control; employee hygiene and health; safety of water and food packing materials; and washing, sanitizing, and packaging.
[2] FSIS Final Rule; FDA Proposed Rule.

- USDA/FSIS develops standards for both shell-egg packers and egg-product processors and provides inspection and enforcement for both.
- FDA and CDC conduct monitoring and surveillance activities. Thus, CDC focuses on human health and FDA on the food supply.

The performance measures that will be used to assess the progress of the plan toward its goal are the numbers of SE cases, isolations and outbreaks reported annually. The data will be collected using the following existing systems:

1. Foodborne Diseases Active Surveillance Network (FoodNet).
2. National Salmonella Surveillance System.
3. National SE Outbreak Surveillance System and Foodborne Diseases Outbreak Surveillance System.

The new data will be compared to the 1998 baseline values of:

- 1.9 cases per 100,000 persons
- 5,900 SE isolations
- 45 SE outbreaks.

12.5 Rapid cooling

Storage temperature has a profound effect on both egg quality and functionality, as well as on microbial growth in shell eggs and on the shell surface. Research has shown that the growth-rate of SE in experimentally-inoculated eggs is reduced as the internal egg temperature decreases (Kim *et al.*, 1989). Furthermore, at a temperature of 7.2 ºC, the pathogen stops replicating, although growth is detected at 10 ºC or higher. Also, Humphrey (1990) demonstrated that, when SE is held at 4–8 ºC, the heat resistance of the organism is reduced, thus making it more susceptible to inactivation by cooking. Based on these and other studies, the current FDA National Food Code and USDA regulation requires that eggs are stored at an environmental temperature of 7.2 ºC or lower. However, current shell-egg processing technologies have limited the ability of the processor to lower the internal temperature with sufficient rapidity.

Cooling rates and requirements for shell eggs vary considerably with egg weight and initial internal temperature. Anderson *et al.* (1992a,b) demonstrated that the temperature of incoming eggs at the processing plant depends on the type of production/processing system and the time of the year. In off-line plants, eggs are collected, packed on flats, palletized and stored for subsequent transport to the processing facility. For in-line plants, eggs are conveyed directly from the layer house to the adjacent processing plant, where they are processed and packaged immediately. In each of these operations, egg temperatures can vary considerably. Anderson *et al.* (1992a,b) reported that eggs having initial temperatures of 16–20 ºC from off-line systems and 23–29 ºC from on-line systems had final, internal temperatures of 26 ºC and 34 ºC, respectively, following processing. The internal temperature of the eggs actually increased

during the processing stages by 6–10 ºC for off-line eggs and by 5–11 ºC for on-line eggs. After processing, the eggs were palletized and placed in a cooler. Cased eggs located on the outside of the pallet reached ambient air temperatures after 73 hours, whereas eggs located in the center of the pallet required 142 hours or more. The authors also noted that each of the processors surveyed in this study experienced problems in maintaining their cooler temperatures.

In April 1990, the Conference for Food Protection recommended that concerns about the possibility of SE in eggs be communicated to all health and food protection agencies and to the food industry. The FDA utilized the Model Food Codes Interpreted Program and placed eggs on the potentially hazardous foods list in August 1990. The designation 'potentially hazardous' means that food regulators can require proper refrigeration and cooking for any listed foods. Two specific requirements for foods on this list that are directly related to the refrigeration of shell eggs were (i) shell eggs must be received at or below refrigeration temperature (7.2 ºC in most states), as specified by the relevant law governing eggs in wholesale distribution, and (ii) shell eggs received in compliance with laws regulating the food during shipment from the supplier must be cooled to 5 ºC or below within four hours (Anderson *et al.*, 1992a).

While the intention of this regulation was to protect public health, it was assumed that an internal temperature of 5 ºC was attainable with current processing and storage technologies. At the time the regulation came into force, however, it was clearly evident that existing egg-cooling methods were unable to cool eggs to 5 ºC within four hours (Anderson *et al.*, 1992a; Czarick and Savage, 1992). The solution was either to change the way eggs were processed or to develop new cooling technologies.

Henderson (1957) demonstrated that forced-air ventilation of palletized eggs produced cooling times close to that of cooling individual eggs. Thompson *et al.* (2000) arranged a 30-case pallet of eggs so that a fan operating at 1000 cubic feet per min drew 4.4 ºC air through openings in the cases. The eggs were cooled to less than 7.2 ºC within one to three hours. It was also mentioned that this cooling system could be used in an existing cold storage room, with little additional investment. Using cryogenic, liquid carbon dioxide, Curtis *et al.* (1995) evaluated the effects of rapid cooling on egg quality and external and internal microbial populations of *Pseudomonas fluorescens*. Eggs exposed to a −51.1 ºC CO_2 environment for three min continued to cool after packaging and reached 7.2 ºC within 15 minutes. The process maintained or even improved egg quality (increase in Haugh units and greater vitelline-membrane strength and resistance to rupture) and shelf-life and did not increase the incidence of shell cracking (Curtis *et al.*, 1995; Hughes *et al.*, 1999; Jones *et al.*, 2002). Further refinements of the cooling apparatus and process resulted in individual eggs reaching the target internal temperature in 80 sec, after exposure to a −56.7 ºC CO_2 environment. Moreover, the internal *P. fluorescens* populations were significantly lower on cryogenically-cooled eggs, compared to eggs cooled by traditional methods. Keener *et al.* (2004) explored cooling rates and CO_2 uptake in commercially processed shell eggs, using a commercial prototype of a

cryogenic (CO_2) egg cooling system. At −70 ºC, the eggs cooled to 7 ºC in 45 min. The uptake of CO_2 into the albumen was significantly greater for cryogenically-cooled eggs (2.11 mg/g) than in those cooled by a traditional method (1.81 mg/g), measured immediately after processing. This difference was still evident in the cryogenically-chilled eggs after ten weeks of refrigerated storage (1.75 and 1.60 mg/g, respectively).

12.6 Pasteurization technologies

12.6.1 Historical background

The storage stability and safety of refrigerated egg products are closely linked to the microbiological quality of the final, packaged product at the time it enters the distribution chain. The primary means of ensuring the microbiological safety of liquid egg products is the use of appropriate pasteurization processes. The term 'pasteurization' refers to the heat treatment of foods at temperatures below those needed for complete sterilization (Banwart, 1981). As a result of product pasteurization, some microorganisms are inactivated, some may be attenuated (i.e. sublethally injured), while bacterial spores may be stimulated to germinate (Banwart, 1981). In the current regulatory context, minimum requirements for pasteurization processes have been designed to destroy certain target microbial pathogens in specific foods. Enhanced microbial shelf-life is an additional benefit of pasteurization, provided that the spoilage organisms present in the raw product are relatively heat-sensitive or are prevented from multiplying through the use of controlled refrigeration, freezing or other means of preservation.

Pasteurization of liquid egg products was first used commercially in the USA in 1938, primarily as a means of extending the shelf-life of frozen, liquid egg products (Cunningham, 1986). A small, gradual increase in the number of companies using thermal treatments for liquid egg occurred in the 1940s and 1950s. The early literature on batch pasteurization of liquid egg was reviewed by Brant *et al*. (1968). At present, most egg processors in the USA utilize continuous egg pasteurization processes. In 1966, regulations promulgated by the USDA and the FDA regarding the incidence of *Salmonella* in processed eggs products, made liquid pasteurization virtually mandatory (Stadelman and Cotterill, 1986). Eventually, the Egg Products Inspection Act of 1970 brought egg pasteurization requirements in the USA under more uniform regulatory control (Elliot and Hobbs, 1980).

12.6.2 Egg pasteurization and *Salmonella*

Current USDA regulations stipulate that liquid, frozen and dried whole-egg, yolk and white must be pasteurized or otherwise treated to inactivate all viable salmonellas (Banwart, 1981). It is important to note that *Salmonella* is the only bacterial pathogen specifically addressed within the context of these regulations (USDA, 1969). A limiting factor in the development of egg pasteurization

processes is the fact that the time-temperature combinations needed to inactivate salmonellas in egg products are at or near those that adversely affect the physical and functional properties of the egg proteins (Elliot and Hobbs, 1980). USDA-mandated egg pasteurization specifications, listed in the Code of Federal Regulations, Title 7, Section 59.570, require that every particle of egg be held for at least a specified time and temperature to 'assure complete pasteurization' (subsection b) and to produce 'a *Salmonella*-negative product' (subsection c) (Anon., 1995).

The pasteurization requirements prescribed by the USDA vary according to the type of egg product, due to the susceptibility of certain egg fractions (e.g. unsupplemented egg white) to heat-induced protein denaturation. Current, minimum time-temperature combinations for the average particle range from 6.2 min. at 55.6 ºC (for unsupplemented albumen) to 3.5 min. at 63.3 ºC (for salted whole egg, sugared yolk, or salted yolk) (Anon., 1995). The liquid egg pasteurization requirements of a number of Asian and European countries were summarized by the International Commission on Microbiological Specifications for Foods (Elliott and Hobbs, 1980). For liquid whole-egg (LWE), these processes range from 2.8 min at 61.7 ºC (Northern Ireland) to 9.0 min at 65.2 ºC (Germany). The predicted lethality of such processes relates only to *Salmonella*. In the USA, a 3.5 min holding time at 60 ºC is said to yield a nine-log (9-D) reduction in the viable *Salmonella* population (where 1-D results in a 90% reduction in the target population) in the average particle of LWE. Under laminar flow conditions, a 9-D process for inactivation of salmonellas would be equivalent to only a 4.5-D process within the fastest-moving particle of egg. The time-temperature combinations prescribed in 1966 for other liquid egg products (Table 12.3) were reportedly designed to provide 'approximately equal pasteurization effectiveness' (USDA, 1969).

In recent years, there has been a renewed interest in defining more accurately the microbiological safety of current USDA-mandated pasteurization processes. Although the implementation of uniform pasteurization requirements for liquid egg products has clearly improved consumer safety, experimental results that are included in the findings of Palumbo *et al.* (1995) and Schuman *et al.* (1997a) indicate that the relative lethality of various liquid-egg pasteurization processes does differ relative to that predicted for *Salmonella* serovars in LWE. This issue represents an active area of egg safety research and an ongoing issue for regulatory policy debate.

12.6.3 Egg pasteurization and shelf-life extension

In addition to providing a margin of safety in relation to *Salmonella*, a secondary benefit of liquid-egg pasteurization is an extension of the refrigerated shelf-life of the product. As noted previously, conventional pasteurization processes do not render the product sterile. Vegetative and sporeforming microorganisms that survive pasteurization may cause liquid egg to spoil during storage, handling and distribution. Furthermore, microbial contaminants capable of spoiling the

Table 12.3 Requirements for conventional pasteurization of liquid egg products in the USA (FDA, 2002; USDA, 1980)

Liquid egg product	Minimum temperature (ºC)	Minimum time (min)
Albumen (without use of chemicals)	56.7	3.5
	55.6	6.2
Whole egg	60.0	3.5
Whole-egg blends (less than 2%	61.1	3.5
added non-egg ingredients)	60.0	6.2
Fortified whole egg and blends (24–38%	62.2	3.5
egg solids, 2–12% added non-egg ingredients)	61.1	6.2
Salted whole egg (2% or more salt added)	63.3	3.5
	62.2	6.2
Sugared whole egg (2–12% sugar added)	61.1	3.5
	60.0	6.2
Plain yolk	61.1	3.5
	60.0	6.2
Sugared yolk (2% or more sugar added)	63.3	3.5
	62.2	6.2
Salted yolk (2–12% salt added)	63.3	3.5
	62.2	6.2

product may be introduced during post-pasteurization handling (Ball *et al.*, 1987). Pasteurized LWE and whole-egg blends currently constitute the main liquid egg products produced in the USA (USDA, 1992). The shelf-life of conventionally-pasteurized LWE is relatively short, ranging from 12 days at 2 ºC to 5 days at 9 ºC (York and Dawson, 1973). Because of such shelf-stability limitations, liquid-egg processors have relied upon either rapid (<14 days) transport and use requirements for the refrigerated product, or frozen storage and distribution (Ball *et al.*, 1987). While extending shelf-life, the process of freezing and thawing whole-egg products causes several undesirable physico-chemical changes, including gelation of the yolk proteins, phase separation, color changes and increased viscosity (Cotterill, 1986). The functional properties of whole-egg products may also be diminished as a result of frozen storage (Jordan *et al.*, 1952; Ijichi *et al.*, 1970).

12.6.4 Ultra-pasteurized, aseptically-packaged LWE

In 1987, Ball and coworkers documented the development of ultra-pasteurization processes (i.e. heating at >60 ºC for <3.5 min.) which, when coupled with aseptic processing and packaging, yielded a product with a shelf-life of 3–6 months at 4 ºC (Ball *et al.*, 1987). At present, commercially-treated product has a code-dated shelf-life of 10 weeks at 4 ºC (Giese, 1994). The extended shelf-life of this non-sterile product is dependent on the use of raw LWE of excellent, initial microbiological quality, unique high temperature-short time thermal treatments, packaging of the product using an aseptic filler within a

sterile zone, and maintenance of proper refrigeration temperatures (1–4 ºC) throughout distribution and retail storage. In 1994, 175 to 200 million pounds of extended shelf-life product were produced in the USA, using these ultra-pasteurization and aseptic packaging technologies (Giese, 1994). Ultra-pasteurized egg products offer a number of advantages over both frozen liquid egg and shell eggs, including consistent product quality, convenience and portion control, storage-space savings and economy in terms of time- and labor-savings, and elimination of the need for frozen distribution (Giese, 1994).When compared with conventionally-pasteurized egg, the ultra-pasteurized product offers enhanced microbiological shelf-stability (at 1–4 ºC) and a greater assurance of safety from *Salmonella*. In addition, achieving a predicted 9-D inactivation of the bacterial pathogen *Listeria monocytogenes* was one of the design criteria used by Ball and coworkers in the development of egg ultra-pasteurization processes (Ronk, 1989). While conventional pasteurization represents only a 2.1–2.7-D process with respect to inactivation of *L. monocytogenes* (Foegeding and Leasor, 1990), the ultra-pasteurization processes of Ball *et al.* (1987) reduced populations of *Listeria* by at least 6.7–>7.3-log units (Foegeding and Stanley, 1990).

12.6.5 Preservation of liquid egg white

Of all the egg components, liquid egg white (and conalbumin in particular) is the most sensitive to heat denaturation. Pasteurization of unsupplemented egg white (pH 9.0) at temperatures of at least 60 ºC produces an unacceptable increase in product viscosity and a decrease in foaming and cake-making functionality (Burley and Vadehra, 1989). Even minimum pasteurization requirements in the USA for unsupplemented egg white (56.7 ºC for 3.5 min.) may markedly increase the whipping time necessary to prepare meringue (Elliott and Hobbs, 1980).

Adjustment of albumen pH

An adjustment of the pH of egg white from its usual level near pH 9 to pH 6.5–7.0 increases the stability of both the albumen proteins and *Salmonella* to heat, but less so for *Salmonella* (Elliott and Hobbs, 1980). In order to maximize the lethality of egg-white pasteurization against salmonellas, either the pH of the albumen should be monitored (for each lot) and the necessary time-temperature adjustments made, or the pH should be adjusted, using a food-grade acidulant, to a level appropriate to the process (Cunningham, 1986). Current US egg pasteurization regulations do not specifically address the issue of albumen pH (Anon., 1995). The Missouri Agricultural Experiment Station has recommended a range of pasteurization temperatures for liquid egg white, as a function of pH. Based on a 0.5 min pre-heating temperature of 48 ºC and a 3.5–4.0 min holding period, the proposed albumen pasteurization temperatures included 54.2 ºC at pH 9.6, 56.6 ºC at pH 9.0, and 59 ºC at pH 8.4 (Cunningham, 1986). By adjusting the pH of liquid egg white to pH 7 with lactic acid and adding small amounts of

aluminum sulfate (which satisfies the chelating power of conalbumin, thereby increasing its heat stability), liquid albumen may be pasteurized at 60–62 ºC for 3.5–4 min. (Lineweaver and Cunningham, 1966; Elliott and Hobbs, 1980). Total soluble protein losses are generally <1%; however, whipping time is still increased and the addition of whipping-aid additives (e.g. methyl citrate, triacetin) is usually recommended (Lineweaver *et al.*, 1967).

Because salmonellas are more heat-sensitive in albumen at alkaline pH values, several researchers have proposed the addition of ammonium hydroxide to increase the pH of egg white to 10.5 or above. Although this pH adjustment provides adequate destruction of *Salmonella* at temperatures below approximately 54 ºC, it is generally recommended only for liquid albumen destined to be spray-dried, at which time the ammonia present will be lost by volatilization (Cunningham, 1986).

Other chemical additives, processing adjuncts and modified pasteurization processes

Hydrogen peroxide has been shown to eliminate *Salmonella* from egg white at room temperature (Ayres and Slosberg, 1949). Armour and Company hold a patent on a hydrogen-peroxide process for egg-white pasteurization at temperatures below 54 ºC (Lloyd and Harriman, 1957). In this process, the product is treated as follows:

1. Liquid egg white is heated to 52–53 ºC and held for 1.5 min.
2. Sufficient hydrogen peroxide (0.5 lb per 100 lb of egg white in a 10% solution) is added to yield a final concentration of 0.075–0.10% in the blended albumen.
3. The hydrogen peroxide is allowed to react at this temperature for 2 min.
4. The egg white is cooled rapidly to 7 ºC and the excess hydrogen peroxide is removed by the aseptic addition of catalase (Baker and Bruce, 1994).

Standard Brands modified the process by injecting a 10% solution of hydrogen peroxide into the egg white (0.875 lb per 100 lb of egg white) before it entered the heating section (USDA, 1969). The mixture is heated to 51.7 ºC for 3.5 min., cooled and catalase added to remove residual hydrogen peroxide. Chemical chelating agents have also been shown to enhance the inactivation of salmonellas in liquid egg white. Garibaldi *et al.* (1969b) reported that supplementation of raw egg white (pH 8.9) with 5 mg/ml of disodium ethylenediamine tetraacetic acid (EDTA) resulted in almost a 4-log reduction in *S.* Typhimurium TM-1 after 8 days of storage at 2 ºC and a >6-log reduction within 28 days. In egg white stored at 28 ºC, the presence of EDTA at 7 mg/ml caused a >6-log reduction in only 10–24 hours. The chelating agent used may have enhanced the sensitivity of salmonellas to the lytic activity of egg-white lysozyme, as has been demonstrated for other antimicrobial agents (Stevens *et al.*, 1991). The use of EDTA as a thermal-processing adjunct also appeared promising. When egg white was supplemented with EDTA (7 mg/ml), decimal reduction times for *S.* Typhimurium TM-1 at 52.5 ºC were reduced by a factor of 3–4 (Garibaldi *et al.*, 1969b). Kohl (1971)

reported that the addition of 0.5–0.7% sodium polyphosphate to liquid egg white (pH 9.5) reduced the heat resistance of *Salmonella* at 50–54 ºC by a factor of 3.7–8.0. Polyphosphates also helped preserve the functional properties of egg white pasteurized for 3.5 min. at 52.2–55 ºC.

The vacuum process is another method of enhancing the microbial lethality of egg-white pasteurization. In this USDA-approved method, a high temperature – short time plate pasteurizer is equipped with a chamber that generates a vacuum (17–20 inches Hg) in the liquid albumen, before it is heated at 57 ºC for 3.5 min (Baker and Bruce, 1994). An important function of the vacuum chamber is to remove air pockets in the albumen and thereby to provide more rapid, uniform heating to the target pasteurization temperature and less 'cooking' of egg white on the plates.

12.6.6 Preservation of LWE and liquid yolk

In comparison with liquid albumen, blended whole-egg is far less sensitive to thermal damage during pasteurization, although *Salmonella* is more heat-resistant in yolk. Thus, higher pasteurization temperatures are possible for yolk products. Following blending, iron from the yolk satisfies the chelating capacity of conalbumin, thereby stabilizing it (Elliott and Hobbs, 1980). Liquid egg-yolk is also relatively heat-stable, but is difficult to process in a continuous manner, because of its high viscosity. Due to the relative heat-stability of LWE and yolk, conventional pasteurization temperatures for unsupplemented products range from 60 to 61.1 ºC (Anon., 1995). For salted and sugared yolk, the pasteurization temperature is 62.2–63.3 ºC.

12.6.7 Extended shelf-life, liquid-egg substitutes

Liquid-egg substitutes represent another category of extended shelf-life products. These products were developed nearly 20 years ago to simulate the features of LWE, yet with little or none of the cholesterol or fat normally present in yolk or whole egg. Egg substitutes consist primarily of liquid egg-white plus a variety of non-egg ingredients (e.g. non-fat dried milk, vegetable oils, emulsifiers, stabilizers, gums, artificial color, vitamins, minerals) that mimic, to an extent, the appearance, flavor and texture of LWE (AEB, 1994). These products are pasteurized and filled into a variety of retail packages, generally in a non-aseptic manner. The finished product has a pH of 6.4–8.0 and a code-dated, refrigerated shelf-life of 8–12 weeks at 1–4 ºC (Ball, 1995).

12.6.8 In-shell pasteurized eggs

As previously discussed, shell eggs may become contaminated with SE by two possible routes: via external contamination and passage of the organism through the shell, or by a transovarian transmission route (Board, 1966). Recent SE outbreaks have generally involved Grade A eggs that have met both state and

local requirements for shell quality and have undergone washing with disinfectants (St Louis *et al.*, 1988). Although many different *Salmonella* serovars have been isolated from the surfaces of eggshells, only SE has been isolated from the contents of intact eggs, using currently-accepted, aseptic sampling methods (Humphrey, 1994). These factors imply that the transovarian route of SE transmission is the more plausible cause of shell-egg contamination.

Based on epidemiological evidence, Gast and Beard (1992) suggested that egg-associated, human SE outbreaks are generally the result of a series of three independent events. First, SE-contaminated eggs must be produced by infected hens. Second, contaminated eggs must be subjected to food-handling practices that permit multiplication of SE to infectious levels. Third, SE-contaminated eggs must be undercooked or consumed raw. Many products such as Caesar salad dressing, hollandaise sauce, eggnog and homemade ice cream have been implicated in SE outbreaks, because, typically, they receive little or no heat treatment prior to consumption (St Louis *et al.*, 1988). Moreover, certain traditional egg-cooking practices (i.e. 'sunny-side' frying, soft poaching, marginal hard-cooking) may be inadequate to kill *Salmonella* in eggs (St Louis *et al.*, 1988).

Long-term control of SE infections will require an examination of the ecology of the causative organism in poultry flocks and may depend, ultimately, on either its elimination from flocks or on the pasteurization of shell eggs and liquid egg. During the 1940s and 1950s, thermal treatment of shell eggs to prevent any embryonic growth, reduce the incidence of spoilage during long-term storage and maintain internal quality received considerable research attention. Stadelman (1995) presents a concise review of that research, with particular attention to the practice of 'thermo-stabilization', a patented process (Fung, 1947), whereby shell eggs are placed in heated water or oil to extend storage life and prevent microbial spoilage. Hou *et al.* (1996) documented the feasibility of combining water-bath and hot-air processing to reduce viable SE populations within inoculated shell eggs. In a related study, Schuman *et al.* (1997b) evaluated the effects of water-immersion heat treatments on the inactivation of SE within intact shell eggs. Six pooled strains of SE (*ca* 3×10^8 cfu per egg, inoculated near the center of the yolk) were completely inactivated within 50–57.5 min. at a water-bath temperature of 58 ºC and within 65–75 min. at 57 ºC (an 8.4–8.5-D process). Haugh unit values and albumen whip-times increased during heating, although yolk index and albumen pH values were unaffected. The authors concluded that broken-out whole-egg or yolk from immersion-heated shell eggs could provide *Salmonella*-free ingredients for the preparation of a variety of minimally cooked foods for consumers and food-service operators.

12.6.9 Non-thermal 'pasteurization' technologies

Irradiation

In the scientific literature, investigators have developed or evaluated a variety of non-thermal approaches to preserving liquid-egg products. The use of ionizing

radiation as a means of inactivating salmonellas in liquid-egg products was first investigated over 40 years ago. Procter *et al.* (1953) reported that, when high-voltage cathode rays (electron beams) were used to treat LWE, the average dose required to yield a 7-D reduction in viable salmonellas was 250 krad for *S.* Typhimurium and *S.* Paratyphi. In a follow-up study, Grim and Goldblith (1965) found that radiation-induced off-flavors were detected by trained taste panels when LWE was gamma-irradiated at doses between only 9 and 30 krad. The intensity of such off-flavors was reportedly reduced when volatiles were liberated during subsequent heating (e.g. in the production of dried whole-egg, scrambling of eggs, baking of sponge cakes).

Electromagnetic and ionizing radiation (e.g. electron beams, gamma rays) have been evaluated as non-thermal means of pasteurizing liquid egg-white (Burley and Vadehra, 1989). Irradiated egg white was reported to be of satisfactory quality in many respects, and radiation processing offers the advantage of being applicable to frozen products. The primary disadvantages involve questions regarding consumer acceptance of irradiated foods in general, and the occurrence of 'off'-flavors (volatiles), sometimes produced by pasteurizing doses (Cunningham, 1986).

Gamma radiation has excellent penetrative power that is particularly important for frozen egg products. Off-flavors have been detected in gamma-treated egg yolk-containing products, but were readily eliminated by spray drying (USDA, 1969). Kijowski *et al.* (1994) reported that gamma irradiation of frozen whole-egg at 2.5 kGy (providing a 0.39 kGy D-value) had no adverse effect on functional or sensory properties. In the case of electron-beam irradiation, no radioactivity or radioactive waste products are generated when the accelerator is activated. The only drawback is its limited penetrative power for foods (3 cm at a beam of 8.1 kW). Wong *et al.* (1996) recorded a 7-log reduction in *S.* Typhimurium per ml of liquid egg-white, following a 2.5–3.3 kGy dose of electron-beam irradiation. It was observed that the irradiated product had 47% less foam loss and a more stable viscosity than thermally-pasteurized material.

Schaffner *et al.* (1989) documented the inactivation of SE in LWE subjected to heating, irradiation or thermo-radiation (combined heat and radiation). Thermo-radiation caused a greater reduction in *Salmonella* than either heat (at 50 or 60 ºC) or radiation (gamma irradiation at 3.9 krad/min) alone. Although several deficiencies were apparent in the methods used for determining thermal resistance, D-values for a single strain of SE were reduced by an average of 34%, when irradiation was combined with heating at 50 or 60 ºC.

The Food and Drug Administration recently announced new rules allowing the irradiation of fresh shell eggs in order to reduce *Salmonella*, including SE (Plummer, 2000). Notification of the rule-making was published in the July 21, 2000 Federal Register. The FDA found that there was little change in levels of individual fatty acids, or in the structure, digestibility or biological value of protein, when shell eggs were treated with ionizing radiation up to 3 kGy. According to research studies, practicable doses will reduce the level of

Salmonella by 10–10,000 fold. However, the FDA emphasized that this process alone would not achieve a 5-log reduction. Moreover, irradiation of eggs may slightly change the viscosity of the eggs, making the white more milky and runny. The irradiated eggs must be labeled in accordance with 21 CFR 179.26. The changes induced can make the egg less effective in some recipes, such as that for angel food cake. Consumers should take this trade-off into account in deciding whether to purchase irradiated eggs.

Serrano *et al.* (1997) determined the radiation sensitivity of five SE strains, inoculated either onto the surface or inside whole shell eggs. The eggs were irradiated at doses of 0, 0.5, 1.0 and 1.5 kGy. A minimum dose of 0.5 kGy was sufficient to eliminate all five strains from the surface of eggs; however, the same strains were more resistant to irradiation when located internally. The ATCC 13076 strain was significantly more sensitive to irradiation, with a D-value of 0.32 kGy, than the other four, which were of animal origin. Irradiation D-values for the latter ranged from 0.39 to 0.41 kGy. On the basis of the D-values obtained, it was reported that a dose of 1.5 kGy should be sufficient to reduce *Salmonella* counts by approximately 4 log units in both shell eggs and liquid egg. The findings also indicated that the color and thermal characteristics of the material were unaffected by a 1.5 kGy dose.

Ultrasonic waves

The potential of ultrasonic waves to inactivate salmonellas in several liquid systems was evaluated by Wrigley and Llorca (1992). In both skim milk and LWE, an exposure time of 30 min (at 40 or 50 ºC) was required to reduce counts of *S.* Typhimurium by more than 0.5 log. Reductions in *Salmonella* levels in LWE treated for 30 min. at a constant temperature of 50 ºC ranged from 1 to 3 log cfu/ml (mean reduction = 1 log cfu/ml). Soluble protein concentrations in ultrasonicated and control samples of LWE were not significantly different.

Pulsed electric fields

The application of short-duration, high-intensity pulsed electric fields (PEF) is another promising technology for non-thermal pasteurization of liquid foods. A review of the literature on this topic was published by Qin *et al.* (1995). The bactericidal effect of high-energy electric fields has been studied for over 30 years. Most research indicates that microbial inactivation is due to the electric field itself (via extensive, irreversible electroporation of the cell envelope) and not due to products of electrolysis or temperature increases (Qin *et al.*, 1995). In typical PEF processes, a pumpable food product (e.g. fruit juices, fluid milk, soups, gravies, liquid egg) is passed through a treatment chamber consisting of two electrodes, through which an electrical power system delivers pulses of a few microseconds. By using multiple PEF exposure cycles and a temperature control system, the temperature of the fluid being treated is maintained between 40 and 55 ºC (Qin *et al.*, 1995). When fresh, raw LWE was subjected to 10 pulses (2 microseconds/pulse, with a peak field-strength of 35 kV/cm), a product was obtained with a shelf-life of 28 days at 4–6 ºC (i.e. approximately double the

shelf-life of conventionally-pasteurized LWE). While the flavor of scrambled eggs prepared from PEF-treated LWE was acceptable, changes in the viscosity and color of the liquid product were noted during refrigerated storage (Qin *et al.*, 1995).

Glucose oxidase

Glucose oxidase (GOX) is an enzyme commonly used to stabilize liquid egg-white by converting glucose to D-gluconic acid, thereby limiting the potential for Maillard browning reactions and color deterioration during storage of the dried material. The reaction of GOX with glucose also produces hydrogen peroxide, a chemical that is bacteriostatic or bactericidal to a wide range of microorganisms. Dobbenie *et al.* (1995) reported that the addition of 0.5 units of GOX and 0.005 g of glucose per ml of fresh, raw LWE completely inactivated SE, *Micrococcus luteus and B. cereus* (vegetative cells, inoculated at 10^5 cfu/ml) after 7–12 days at 7 ºC. This treatment was only bacteriostatic to *P. fluorescens* in LWE stored at 7 ºC for up to eight days. The combination of 0.5 U GOX/ml and 0.005 g of glucose/ml extended the lag phase of the natural microbial flora in raw LWE stored at 7 ºC by six days, relative to unsupplemented controls (Dobbenie *et al.*, 1995).

Nisin

In the history of the egg processing industry, a wide variety of food additives (e.g. carbohydrates, hydrocolloids, organic acids, vitamins) has been evaluated and used to enhance the functional and nutritional properties of liquid egg products. In practice, only relatively high sodium chloride concentrations (i.e. $\geq$5%, mainly to inhibit gelation during frozen storage) have been shown to substantially inhibit the growth of most spoilage organisms and bacterial pathogens during refrigerated storage of liquid egg (Elliott and Hobbs, 1980; Erickson and Jenkins, 1992).

Addition of the bacteriocin nisin to liquid egg products represents a novel approach to enhancing microbiological safety and shelf-life during refrigerated storage. Several investigators have suggested that the use of nisin or other bacteriocins (e.g. subtilin, tylosin) could allow a reduction in the severity of thermal processes used for various foods (Pflug and Holcomb, 1991). However, the varying susceptibility of microorganisms to nisin (even within a genus or species) and the diversity of organisms that can contaminate foods dictate that a conservative approach is always taken to ensure consumer safety, and other approaches may be preferred. A bacteriocin is defined as a proteinaceous compound (usually a peptide) having bactericidal action against a limited range of organisms that tend to be closely related to the producer organism (Barnby-Smith, 1992). Many bacterial species have been shown to produce bacteriocins or bacteriocin-like substances, and synthesis may provide a survival advantage for the organisms in question within harsh environments. Of all the bacteriocins, nisin has been the most studied, and is the only one that has been exploited commercially for food preservation (Barnby-Smith, 1992). Nisin is a 34-amino

acid pentacyclic peptide that is produced by the dairy starter organism *Lactococcus lactis* ssp. *lactis.* Extensive reviews of the biology, chemistry, toxicology and biosynthesis of nisin have been published by Hurst (1983), Liu and Hansen (1990), Ray (1992) and Hansen (1994).

The antibacterial spectrum of nisin is relatively broad in comparison with other bacteriocins and includes certain members of the following Gram-positive genera: *Bacillus, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Listeria, Micrococcus, Pediococcus* and *Staphylococcus.* In addition, nisin exhibits bactericidal activity against several *Clostridium* spp.

Two publications highlight the potential for nisin to enhance the lethality of thermal processes. Lewis *et al.* (1954) evaluated the effects of 67 antimicrobials on the thermal-resistance of spores of *Clostridium sporogenes* PA 3679, heated at 120.1 ºC in phosphate buffer (pH 7.0). Of these, nisin (560 IU/ml) was the most effective additive, with reductions in D-values of 55–57%.

In a more recent study, Niewiarowicz *et al.* (1980) assessed the impact of nisin (8,000 IU/g) and sodium polyphosphate (0.5%) on the thermal-resistance of *Staph. aureus* and *Escherichia coli* in liquid albumen and in LWE. Samples of the material were supplemented with polyphosphate and/or nisin, inoculated with the target organism (2×10^6 cfu/g), dispensed in 20 ml amounts into glass beakers and heated with constant stirring in a pre-heated water bath. The presence of polyphosphate, with or without nisin, enhanced the inactivation of *Staph. aureus* in albumen (pH 6.9 and 9.1), heated at 55 or 57 ºC for 1.5 min. Nisin alone did not accelerate thermal inactivation of the organism in the more alkaline albumen at either temperature tested. At pH 6.9, nisin enhanced the thermal destruction of *Staph. aureus* in albumen held at 57 ºC, but not at 55 ºC. Nisin was not evaluated for activity against the Gram-negative organism, *E. coli*; in most cases, this organism was inactivated (i.e. a 6.3-D process) by heating at 55 or 57 ºC for 1.5 min., regardless of the presence of sodium polyphosphate. In the case of LWE (pH 7.5) heated at 56, 58, or 60 ºC, the value of the thermal-processing adjuncts was most evident in egg heated at 56 ºC for two min. This heat process reduced viable *Staph. aureus* populations by 0.4 log (LWE control), 2.8 logs (LWE + nisin), 3.0 logs (LWE + polyphosphate) and 3.8 logs (LWE + nisin + polyphosphate). In LWE, heating at 56 ºC for two min represented a 0.4-D process (LWE control) and a 3.1-D process (LWE + polyphosphate) for the *E. coli* challenge inoculum.

Similar studies that evaluated lower, more cost-effective levels of nisin as a thermal-processing adjunct for liquid egg products would be worthwhile. Of particular interest would be the effects of such processes on important egg-associated pathogens (e.g. *Salmonella* serovars, *L. monocytogenes, B. cereus*). In using nisin for this purpose, the influence of product pH on the solubility and stability of nisin during heat processing and subsequent storage are important considerations (Pflug and Holcomb, 1991). These and other questions were addressed by Schuman (1996), who sought to evaluate the effects of sublethal concentrations of nisin on the thermal resistance of *L. monocytogenes* in raw LWE. In this study, dilute solutions of purified nisin in 0.02 N HCl were added

to samples of inoculated LWE (pH 6.5) to yield nisin concentrations of 0, 20, 40, or 60 IU/ml (marginal, minimum inhibitory concentration for *L. monocytogenes*). Following either an 18–20 hour or 30 min. incubation period at 4 ºC, the cells were then heated in sealed capillary tubes immersed in a pre-heated water bath at 60 ºC and D-values calculated from the linear portions of the resulting survivor curves. The presence of nisin reduced the D_{60}-value of *L. monocytogenes* (initially 2.4 min) by 30% (20 IU/ml), 32% (40 IU/ml) and 27% (60 IU/ml), following the 18–20 hour exposure to nisin, and similar results were obtained after only 30 min. The heat-sensitizing effect of nisin could be attributed to structural destabilization of the *Listeria* cell wall (i.e. sublethal injury). Alternatively, heat-induced injury may have made a proportion of the cells more susceptible to the biocidal activity of nisin.

As well as being used as a processing adjunct to decrease the heat-resistance of bacteria in liquid egg products, nisin has also been applied to LWE to inhibit growth and survival of *L. monocytogenes* (Schuman, 1996; Sheldon and Schuman, 1996). In these studies, the addition of nisin (1000 IU/ml) to pH-adjusted, ultra-pasteurized LWE reduced *L. monocytogenes* populations by 1.6–>3.3 log cfu/ml and delayed (pH 7.5) or prevented (pH 6.6) growth of the pathogen throughout 8–12 weeks at 4 or 10 ºC. Bioactive nisin was detected in the LWE at both pH values throughout incubation at 4 ºC. In subsequent experiments, *Listeria* reductions of >3.0 log cfu/ml were achieved within 24 hours for both LWE and broth plus nisin (500 IU/ml) at pH 6.6, but not at pH 7.5, and antilisterial activity was enhanced, when nisin was added as a solution rather than in dry form.

The use of nisin to extend the pathogen-free shelf-life of LWE products held under chill conditions represents another novel application of this food ingredient. For example, Delves-Broughton *et al.* (1992) evaluated the addition of nisin at 200 IU/ml (5 mg/l) as a means of extending the refrigerated shelf-life of conventionally-pasteurized LWE. Nisin was added before the product was pasteurized according to UK requirements (i.e. 64.4 ºC for 2.5 min.). In two trials, nisin-free controls had a shelf-life of 6–11 days at 6 ºC. In contrast, pasteurized material containing nisin had a shelf-life of 17–20 days. Nisin-supplemented samples were free of detectable *B. cereus*, eventually spoiling from the growth of *Pseudomonas* spp. Control samples underwent an acidic-type spoilage due to the growth of *B. cereus*.

At the same time, a series of trials was conducted at the M. G. Waldbaum Company (Gaylord, USA) in which nisin (at 100–250 IU/g) was evaluated as a biopreservative in commercially-processed, ultra-pasteurized and aseptically-packaged LWE containing 0.15% citric acid (Samimi, 1992). The addition of 250 IU/g of nisin prior to heating yielded a shelf-life of $\geq$12 weeks at 4.4 ºC. Based on a conservative criterion for the end of shelf-life, i.e. an aerobic plate count $>10^4$ cfu/g, regardless of whether sensory defects were detectable, the treated LWE had a storage life of three and 14 days under simulated, abusive storage conditions at 21.1 and 10 ºC, respectively. Because of the small microbial populations present in the raw product and the severity of the thermal

process, even a nisin level of 100 IU/g had some effect in delaying spoilage during shelf-life tests (Samimi, 1992).

12.6.10 Thermal-resistance of bacterial pathogens in eggs and egg products

Salmonella

Because liquid-egg pasteurization processes were designed to ensure inactivation of *Salmonella*, numerous investigators have sought to define the thermal inactivation kinetics for this important pathogen (Elliott and Hobbs, 1980; Humphrey, 1994). The conventional pasteurization process for LWE (i.e. 3.5 min. at 60 ºC for the average particle) was based on the assumption that typical strains of *Salmonella* have a D-value at 60 ºC of approximately 0.4 min. in blended whole egg (USDA, 1969). Despite differences in *Salmonella* serovars/strains and in the experimental procedures used, this assumption has proved to be reasonably accurate, based on kinetic data from a variety of research laboratories.

Representative decimal reduction times for *S.* Oranienburg and *S.* Typhimurium in various liquid egg products are presented in Table 12.4 (Elliott and Hobbs, 1980). The predicted D-value for *S.* Oranienburg in LWE (pH 7.0) at 60 ºC is 0.3 min., when extrapolated from the survival curve of Cotterill *et al.* (1973). Humphrey *et al.* (1990) reported D-values of 0.20 and 0.26 min. respectively for two *S.* Typhimurium strains heated in LWE at 60 ºC. As shown in Table 12.4, *S.* Oranienburg and *S.* Typhimurium were more heat-resistant in plain yolk than in LWE (pH 7), while the organisms were more heat-sensitive in unsupplemented albumen (pH 9.2–9.5) than in whole egg. Similar heat-resistance trends were documented by Humphrey *et al.* (1990) for three strains of SE and two of *S.* Typhimurium. The D-values for the five strains ranged from 0.20 to 0.44 min in LWE heated at 60 ºC (Humphrey *et al.*, 1990). In a similar study of 17 strains of SE (primarily phage type 8), Shah *et al.* (1991) reported that D-values at 60 ºC ranged from 0.20 to 0.52 min. in LWE (mean = 0.32 min.). Even with a D-value of 0.52 min., conventional pasteurization of LWE at 60 ºC for 3.5 min. would provide a predicted lethality of 6.7 log units. Baker (1990) used a continuously-stirred, three-necked flask to assess the thermal-resistance of nine strains of SE in raw LWE. The D-values at 60 ºC ranged from 0.31 to 0.69 min. (mean = 0.42 min.). Based on the above decimal reduction times, a 3.5 min. process at 60 ºC would yield a 5.1–11.3-log reduction in SE.

The published data for thermal-resistance of *Salmonella* in liquid egg-white or yolk have varied widely according to the strains tested, the investigator and the laboratory methods used. For example, Humphrey *et al.* (1990) reported a D-value of 1.0 min. for *S.* Typhimurium heated at 55 ºC in albumen. Using a different strain of the same serovar, Garibaldi *et al.* (1969a) predicted a D-value of only 0.58 min. at 55 ºC. Similarly, for egg yolk, the D-values at 60C reported by Humphrey *et al.* (1990) (D = 0.8 min.) and Garibaldi *et al.* (1969b) (D = 0.4 min) differed substantially for two strains of *S.* Typhimurium. Palumbo *et al.*

Table 12.4 Thermal resistance of *Salmonella* spp. in various liquid egg products (Elliot and Hobbs, 1980)

Test organism	Product	D-value (min) at ºC							z-value (ºC)	Reference
		50	52	54	56	58	60	62		
S. Oranienberg	Whole egg pH 7.0	—	—	4	1.7	—	—	—	4.5	Cotterilll *et al.* (1973)
	Whole egg + 10% sucrose	—	—	25	10	3.5	—	—	4.3	
	Whole egg + 10% salt	—	—	30	12	6	3	—	7.0	
S. Typhimurium	Whole egg pH 5.5	—	—	17	5.5	—	—	—	4.2	Lategan and Vaughn (1964)
	Whole egg + sorbic acid 100 ppm	—	—	14	4.5	—	—	—	4.1	
	Whole egg + β-propiolactone 100 ppm	—	—	11	4.5	—	—	—	4.5	
	Whole egg + benzoic acid 500 ppm	—	—	6.5	2.2	—	—	—	4.1	
S. Typhimurium	Egg yolk	—	—	9.5	3	1.3	0.4	—	4.4	Garibaldi *et al.* (1969b)
	Egg yolk + 10% sucrose	—	—	75	25	10	4	—	4.8	
	Egg yolk + 10% salt	—	—	110	45	17	6	2	4.6	
S. Typhimurium	Egg white pH 9.2	10	3.5	1	—	—	—	—	4.2	Garibaldi *et al.* (1969b)
	Egg white pH 9.2 + 10% sucrose	20	7	2.5	0.8	—	—	—	4.3	
	Egg white pH 7.3 + aluminum salt	—	22	6.5	2.5	—	—	—	4.2	
Mix of 3 strains	Egg white pH 9.5	9	3.5	1.1	—	—	—	—	4.8	Kohl (1971)
	Egg white pH 9.5 + 0.5% polyphosphate	1.1	0.6	0.3	—	—	—	—	4.6	

(1995) reported D-values at 60C of 0.55–0.75 min. for six *Salmonella* serovars heated in unsupplemented, liquid yolk. For five pooled strains, Schuman and Sheldon (1997) reported D-values in raw egg yolk (pH 6.3) and egg white (pH 8.2 versus 9.1) of 0.087 min. (at 62.2 ºC), 0.28 min. (at 60 ºC) in yolk and from 1.0 min. (at 58.3 ºC) to 7.99 min. (at 55.1 ºC) in egg white (pH 8.2).

In summary, the thermal-resistance data discussed above indicate that conventional pasteurization processes for liquid yolk, albumen (pH 9.2) and LWE are predicted to provide minimum reductions in *Salmonella* of 7.75, 6.2 and 5.1 log units, respectively. It should be noted that methodological differences used in assessing the thermal-resistance of salmonellas (and other pathogens) in liquid egg may affect the D-values reported. In addition to the known factors affecting the heat inactivation of microorganisms (e.g. proximate composition of the heating menstruum, moisture content, pH, inoculum concentration, growth conditions), the geometry of the container and its headspace volume, and orientation within the heating bath, may bias the resultant kinetic data (Beckers *et al.*, 1987; Donnelly *et al.*, 1987; Jay, 1992a). The analysis of sub-samples drawn from a single, large heated vessel appears to be particularly unreliable. For example, Dabbah *et al.* (1971) reported exceptionally high D-values of >6.0 min. at 60 ºC, when *Salmonella* serovars were heated in LWE in a 300 ml glass flask, despite continuous stirring. While there remains no general consensus on the best experimental method for such testing, the advantages of using small, sealed and fully-immersed containers have been well documented (Donnelly *et al.*, 1987; Pflug and Holcomb, 1991).

Several investigators have evaluated the efficacy of simulated domestic or food-service cooking methods as means of inactivating SE in Grade A shell eggs. Saeed and Koons (1993) asked individuals who identified themselves as regular egg consumers to cook separate sets of 11 eggs by each of three methods. As expected, the individuals varied considerably in the times they used to cook the egg dishes to their preferred degree of doneness. When the contents of the initial shell eggs were inoculated with 10^1–10^3 cfu per ml of salmonellas, the organisms were detected in 24% of fried eggs, 15% of scrambled eggs and 10% of omelets tested. Baker (1990) reported that internal end-point temperatures of 74 ºC for scrambled eggs and 61–70 ºC for fried eggs were necessary to ensure the inactivation of SE. With respect to boiled eggs, Licciardello *et al.* (1965) and Baker *et al.* (1983) found that, for shell eggs internally inoculated with 10^8–10^9 *Salmonella* cells, the pathogen was effectively inactivated, when the eggs were placed directly into boiling water for a minimum of 7–8 min. While the above procedures provide valuable guidance for safe preparation of fully-cooked egg dishes, there is a need for the egg industry to provide shell eggs with a greater assurance of microbiological safety to retail consumers and the food service industry. Such a development would be particularly valuable if it allowed the safe preparation of egg-containing foods that receive little or no cooking, e.g. soft-boiled eggs, soft-poached eggs, ‘sunny-side’ fried eggs, gourmet sauces, salad dressings and custards.

Listeria monocytogenes

The thermal resistance of *L. monocytogenes* in a variety of foods and microbiological media has been reviewed by Mackey and Bratchell (1989) and Farber and Peterkin (1991). The literature supports the contention that *L. monocytogenes* is among the more heat-resistant non-sporeforming pathogens associated with foods, with a calculated Z_D-value range in most foods and broths of 6.7–6.9 ºC (Mackey and Bratchell, 1989). In the aftermath of the 1983 milk-associated outbreak of listeriosis in Massachusetts, USA, the adequacy of milk pasteurization processes to inactivate *L. monocytogenes* was the subject of numerous publications. In a summary of this research, Mackey and Bratchell (1989) predicted that high-temperature, short-time pasteurization conditions (71.7 ºC for 15 sec.) and vat pasteurization conditions (63 ºC for 30 min.) would achieve 5.2- and 39-D reductions, respectively. These results are noteworthy in that a similar '5-D' lethality standard for *L. monocytogenes* could be considered appropriate for continuously-pasteurized (conventional), liquid egg products.

Foegeding and Leasor (1990) published a study in which D-values were determined for five strains of *L. monocytogenes* in sterile, raw LWE. The experimental units consisted of 0.05 ml samples of inoculated LWE within sealed, glass capillary tubes, which were heated by immersion in a pre-warmed water or oil bath. The D-values for strain F5069, the most heat-resistant isolate tested, averaged 22.6, 7.1, 1.4 and 0.20 min. at 51, 55.5, 60 and 66 ºC, respectively. For the five strains evaluated, minimum pasteurization conditions (60 ºC for 3.5 min.) were predicted to represent 2.1–2.7-D processes with respect to *L. monocytogenes* in whole egg. In another study, Foegeding and Stanley (1990) determined thermal death times (F-values) for *L. monocytogenes* F5069 in raw LWE, using the procedure that involved immersion of sealed capillary-tubes (0.05 ml of liquid egg per tube) referred to above. This *Listeria* strain was eliminated from inoculated samples (5.0×10^6–2.0×10^7 cfu/tube) after 16, 8, 4.5, 1.6 and 0.6 min. at 62, 64, 66, 69 and 72 ºC, respectively.

Bartlett and Hawke (1995) evaluated the heat resistance of *L. monocytogenes* strains Scott A (a clinical isolate) and HAL 975E1 (an egg isolate) in the following five liquid-egg products: LWE, 10% NaCl in whole egg (LWEN), 10% sucrose in whole egg (LWES), 10% NaCl in egg yolk (EYN) and 10% sucrose in egg yolk (EYS). The presence of salt reduced the water activity (a_w) of the products to a greater extent than sucrose; however, all water activity values were >0.91, except for salted yolk ($a_w = 0.867$). Following the use of a submerged, sealed test-tube procedure, survivor curves were obtained and D-values calculated. The relative heat resistance of *L. monocytogenes* in the five products was as follows: heat resistance in EYN > LWEN $\gg$ EYS $\geq$ LWES $\geq$ LWE. These thermal-resistance trends were very similar to those reported by Cotterill *et al.* (1973) for *S.* Oranienburg in liquid egg products. Based on current US egg pasteurization requirements, Bartlett and Hawke (1995) predicted that reductions in *L. monocytogenes* Scott A would range from 0.2 log cycle (for salted egg yolk) to 1.8 log units (for sucrose-containing LWE).

Similar thermal-resistance trends were reported by Palumbo *et al.* (1995),

who determined D-values for six pooled strains of *Salmonella* serovars and five pooled strains of *L. monocytogenes* inoculated into liquid egg-yolk (EY) and various EY products containing added salt and/or sucrose. Both pathogens were more heat-resistant in EY + 10% NaCl than in EY + 10% sucrose, or in plain EY. Based on D-values derived from the use of a submerged, capped test-tube procedure, the lethality of USDA-mandated, conventional egg pasteurization processes was estimated to range from 0.3 log (in EY + 10% NaC1) to 6.1 logs (in plain EY) for *Salmonella*, and from 0.2 log (in EY + 10% NaCl) to 3.3 logs (in EY + 10% sucrose) for *L. monocytogenes*.

In summary, the four studies described above indicate that the margin of safety provided by conventional pasteurization for LWE, plain yolk and products containing added sucrose is not large, especially if *L. monocytogenes* is present in the raw bulk material at levels greater than 100 cfu/ml. In NaCl-supplemented LWE or yolk, conventional pasteurization would be inadequate to inactivate even 10 cfu/ml of *L. monocytogenes*. These findings take on additional significance in the light of a USDA document that details the criteria for approval to produce and market liquid egg products with an 'extended shelf-life' (i.e. >4 weeks at 4.4 ºC) (USDA-AMS, 1992). In order to gain regulatory approval for such products, companies must pasteurize the product at 60 ºC for 3.5 min. If alternative thermal processes are used, the company must provide laboratory data demonstrating that the process yields a 7-log reduction in *L. monocytogenes.* As noted previously, the data of Foegeding and Leasor (1990) and Bartlett and Hawke (1995) clearly demonstrate that a 3.5 min. process at 60 ºC represents only a 1.7–2.7-D *Listeria* inactivation process. Thus, the proposed 7-D lethality requirement appears to be unduly harsh and may not allow production of liquid egg products with acceptable organoleptic and functional quality.

Schuman and Sheldon (1997) determined D-values for *L. monocytogenes* (five pooled strains) in raw, liquid egg yolk (pH 6.3) and egg white (pH 8.2 *vs.* 9.1), using a small amount of material (0.05 ml/sample) and an immersed, sealed capillary-tube procedure. The D-values ranged from 0.58 min. (at 62.2 ºC) to 1.34 min. (at 60 ºC) in yolk and from 2.41 min. (at 58.3 ºC) to 7.59 min. (at 55.1 ºC) in egg white (pH 9.1). Mean z_D-values ranged from 6.06 to 9.43 ºC. In egg white, the heat sensitivity of *Listeria* was enhanced at the higher pH value. USDA-mandated, conventional pasteurization requirements for egg yolk (equivalent to 3.9–4.6-D processes, on the basis of the Schuman and Sheldon (1997) study) are far more lethal to *L. monocytogenes* than are the recommended thermal processes for egg white (equivalent to 0.7–0.8-D processes).

The survival potential of *L. monocytogenes* in eggs heated under simulated food service or domestic conditions has been evaluated in two studies. Using inoculated shell eggs (containing $>5 \times 10^5$ cfu/g of egg contents), Urbach and Shabinski (1955) reported that listerias were isolated from fried eggs cooked in a manner that coagulated the white, but left a 'soft yolk'. In a more detailed study, Brackett and Beuchat (1992) reported that frying inoculated, whole eggs 'sunny-side up', until the albumen was partially coagulated, reduced both low (10^2 cfu/

g) and high (10^5 cfu/g) inocula of *Listeria* by only 0.4 log cfu/g. In contrast, cooking one or three scrambled eggs on a skillet to an internal end-point temperature of 70–73 ºC reduced the lower inoculum by >1.8 log and the higher inoculum by 3.0 log units. The authors concluded that, although it is unlikely that large numbers of listeria would survive such cooking procedures, unless present at >10 cfu/g, it would be prudent for vulnerable individuals to consume only 'thoroughly-cooked' eggs (Brackett and Beuchat, 1992). However, as stated previously, there have yet to be any documented cases of listeriosis linked to the consumption of eggs or egg products.

Aeromonas hydrophila

The documented growth potential of *A. hydrophila* in LWE at 6.7 ºC (slight temperature abuse) highlights the importance of ensuring the adequacy of egg pasteurization/ultra-pasteurization, with respect to inactivation of the organism. To date, only two published studies have addressed the heat resistance of *A. hydrophila* (human clinical isolate) in an egg product. Nishikawa *et al.* (1993) reported that a 4 min. process at 55 ºC was sufficient to inactivate the pathogen, initially inoculated at 10^8 cfu/ml into raw egg-yolk and heated in capped test-tubes in a pre-heated water bath. The authors also demonstrated that *A. hydrophila* was substantially less heat-resistant than a clinical isolate of *S.* Typhimurium, when heated in liquid yolk. Because the study was conducted at only one water-bath temperature (55 ºC, without correction for warm-up time), neither D- nor z_D-values were reported. In the second study, Schuman *et al.* (1997b) sought to characterize kinetically the heat resistance of *A. hydrophila* in raw whole-egg, using a sealed capillary-tube procedure with total immersion. Decimal reduction times for four individual strains of *A. hydrophila* at 48, 51, 54, 57 and 60 ºC were found to range from 3.62 to 9.43 min. at 48 ºC to 0.026 to 0.040 min. at 60 ºC. Two processing plant isolates were more heat-resistant than the culture collection strains. The z_D-values were 5.02–5.59 ºC, similar to those for other, non-sporing bacteria. Although this study indicated that *A. hydrophila* is substantially less heat-resistant than *Salmonella* in LWE, it is important for egg processors to prevent post-pasteurization contamination with *Aeromonas* spp. and other psychrotrophic pathogens, including *L. monocytogenes*. At the time of this review, no other reports were available on the thermal-resistance of *Aeromonas* spp. in other egg products or shell eggs cooked under simulated food service conditions.

Although studies have attempted to characterize the thermal inactivation of *A. hydrophila* in model buffer systems and in skim milk (Palumbo *et al.*, 1987; Condón *et al.*, 1992), each one yielded non-linear (tailing) survivor curves that made analysis of the thermal-destruction data more difficult. The diphasic inactivation curves showed surviving populations of 10^2–10^5 cfu/ml of suspension, long after the initial linear phase of inactivation. For the purposes of calculating D-values, the authors disregarded the tailing portions of the survivor curves, while noting the potential significance of microbial sub-populations of apparently greater heat-resistance than the rest of the inoculum.

Using a buffered peptone system, Stecchini *et al.* (1993) reported that the inactivation of A. *hydrophila* in 9 ml capped test-tubes was a non-linear process, best described mathematically by a complex hyperbolic function.

The straight-line, semi-logarithmic destruction model for microorganisms is among the most important tools used in the food and allied industries to predict thermal process lethality for organisms of public health concern (Pflug, 1987). In a review of the literature on heat destruction of microorganisms, Pflug and Holcomb (1991) reported that, in 40% of cases, the data obtained form an approximately straight-line survivor curve; in another 40%, the data form a curve in which an initial shoulder (concave downward) or dip (concave upward) is followed by the remaining, straight-line portion (Pflug, 1987). Non-linear or multi-phasic curves have been reported for a variety of microbial spores and vegetative cells (Cerf, 1977), including *Salmonella* in LWE (Dabbah *et al.*, 1971). Several authors have reviewed the subject of non-linear microbial inactivation (Moats *et al.*, 1971; Cerf, 1977), yet there remains no consensus as to whether varying responses to lethal temperatures result from genetic or physiological differences among the cells within a 'pure' culture suspension, differences in the mechanism of thermal inactivation, or whether such tails represent artifacts of the laboratory methods.

12.7 Future trends

This review has mainly covered the more traditional, thermal methods for pasteurizing eggs and egg products. However, some attempt has been made to introduce approaches that may enhance the inactivation process, including the addition of chemical additives or adjuncts to egg products (hydrogen peroxide, chelating agents, nisin). In addition, some non-thermal pasteurization processes, such as irradiation, ultrasonic waves, pulsed electric fields, have been considered. Clearly, there are a number of other technologies that are in various stages of development, evaluation and commercialization. The Institute of Food Technologists (2000) published a special supplement that reviewed a number of alternative methods for pasteurizing food products. Although some have already been addressed briefly in this chapter, there are a number of others that have potential for treating shell eggs and liquid egg products, including microwave and radio-frequency processing, ohmic and inductive heating, high pressure processing, high voltage arc discharge, pulsed-light technology, oscillating magnetic fields, ultrasound and pulsed x-rays. Each technology is discussed in great detail in the IFT publication. Moreover, their advantages and disadvantages are considered, including cost, impact on product quality, efficacy against targeted pathogens and need for further research.

As well as optimizing process technology, a much greater effort is needed to control the safety of eggs and their products at the farm-production level and during the primary stages of egg handling and processing. Better management practices are needed in layer houses, including tighter biosecurity measures,

more effective rodent control programs, assurance of *Salmonella*-free chicks and pullets, better monitoring and control of environmental conditions, effective use of vaccines against *Salmonella* and the application of nutritional programs that enhance the innate immune system of the bird against attack by bacterial and viral agents, and prevent intestinal colonization by foodborne pathogens. Moreover, other innovative, on-farm approaches are being developed and commercialized, such as the feeding or spraying of defined 'competitive exclusion' products containing desirable microorganisms that are capable of colonizing the gastrointestinal tract of laying fowl at the expense of pathogens, or the feeding of bacteriophages with the capacity to attack and destroy key pathogens *in situ.*

Ensuring the safety of eggs and egg products is the responsibility of many different individuals, including the producer, processor, distributor, marketing agent, wholesaler, retailer, food service and institutional feeding worker, regulatory agency and consumer, in a 'farm-to-fork' approach. Much of these areas of responsibility will be covered under the pending Federal 'Egg Safety Action Plan' which aims to eliminate human illness from contamination of eggs with SE.

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13

Improving slaughter and processing technologies

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13.1 Introduction

In 1996, the Foodborne Diseases Active Surveillance Network (FoodNet) collected data on nine diseases at several sites within the USA (USDA-FSIS, 1997). Since the start of this program, *Salmonella* and *Campylobacter* have been the leading causes of laboratory-confirmed foodborne illness. During 1997, *Salmonella* (2,204 cases) and *Campylobacter* (3,966 cases) accounted for over 76% of the confirmed, foodborne-related diseases (USDA-FSIS, 1998). In direct comparisons in the USA between *Salmonella* and *Campylobacter*, *Campylobacter* outnumbered *Salmonella* detection ten to one in college students and two to one in the general population (Tauxe, 1991). The continued demand for the production of safe, 'clean' food products remains a high priority for food producers. To meet such a demand, government, universities and industry must continue working together to identify and implement critical control points (CCPs) to reduce foodborne pathogens. Because *Salmonella* and *Campylobacter* are the leading causes of foodborne illness throughout the world, the present chapter focuses on these organisms and considers the problems that arise in commercial poultry processing and their control.

13.2 Contamination risks during slaughter and evisceration of poultry

13.2.1 Feed withdrawal

A standard management practice in commercial broiler production is withholding of feed immediately prior to processing to allow clearance of the

gastrointestinal tract and thereby reduce visible contamination of poultry carcasses (May and Lott, 1990). Although this *ante-mortem* practice of feed withdrawal has been shown to reduce the number of carcasses that are visibly contaminated with feces, the practice does increase the incidence of *Salmonella*- and *Campylobacter*-positive crops (Ramirez *et al.*, 1997; Byrd *et al.*, 1998; Corrier *et al.*, 1999a). Ramirez *et al.* (1997) found that feed withdrawal increased the number of *Salmonella* isolations from crops in both experimentally- and naturally-infected broilers. Similarly, feed withdrawal resulted in more than a doubling of *Campylobacter*-positive crops in commercially-reared broilers, when compared with fully fed birds (Byrd *et al.*, 1998). In relation to *Campylobacter*, broilers without water during feed withdrawal had a significantly higher isolation rate from cloacal samples (17% higher) and a lower isolation rate (5% lower) from the ceca, in comparison with birds given water (Willis, *et al.*, 1996). In a second study, broilers deprived of feed for 12 h, with or without access to water, had a cecal detection rate of 100% (Willis *et al.*, 1996). The results of these studies suggest that crop contamination represents a potential CCP for reducing the entry of *Salmonella* and *Campylobacter* into the processing plant, and that ceca, too, can be a significant source of *Campylobacter* contamination, even when feed is withheld.

Changes in the normal microflora of the crop can be associated with the consumption of contaminated litter and feces by the birds during feed withdrawal and may contribute to an increase in the incidence of pathogens in the crop contents (Corrier *et al.*, 1999a). Indeed, the resident population of lactobacilli in the crop has been shown to compete with coliforms and pathogenic bacteria for both binding sites and nutrients in laying hens (Fuller, 1973, 1977; Fuller and Brooker, 1974; Humphrey *et al.*, 1993). During an eight-hour feed withdrawal period, crop pH increased, while numbers of lactobacilli decreased, with an associated fall in lactic acid concentration (Corrier *et al.*, 1999b). The consumption of pathogen-contaminated litter and a more favorable environment for pathogenic bacteria are likely to be the main causes of the rise in *Salmonella*- and *Campylobacter*-contaminated crops following feed withdrawal. Of additional concern is the fact that conditions in the crops of birds deprived of feed increased the virulence of *Salmonella* by favoring the *in vitro* expression of genes necessary for intestinal invasion (Durant *et al.*, 1999).

13.2.2 Transportation of birds to the processing plant

Broilers that have undergone feed deprivation for four hours or more are caught and transported to the processing plant. During transportation, the birds are exposed to transport coops that may be contaminated with *Salmonella* and *Campylobacter* (Rigby and Petit, 1980; Hoop and Ehrsam, 1987; Stern *et al.*, 1995). Stern *et al.* (1995) found that transportation increased the incidence of *Campylobacter*-positive birds (56%) when compared to pre-transport birds (12.1%). Furthermore, mean total numbers on carcasses increased from 2.71 to 5.15 log cfu (Stern *et al.*, 1995). However, transportation does not seem to have a

uniform effect in increasing the frequency of *Campylobacter* contamination in all plants studied, and exceptions have been noted (Jones *et al.*, 1991a).

Salmonella-positive cecal carriers were found to increase during experimental shipping conditions from 23.5% (controls) to 61.5% (Rigby and Pettit, 1980). Similarly, Jones *et al.* (1991b) found that 33% of unwashed transport coops were contaminated with *Salmonella*, although the broilers transported in this study were negative for *Salmonella*, according to results obtained from cloacal swabs.

The presence of foodborne pathogens in transport coops is indicative of a potential source of external contamination and internal colonization of the birds. Mead *et al.* (1994) inoculated transport coops with a marker strain of *Escherichia* coli and showed that it survived normal cleaning with chlorinated water. These researchers also found that 50% of the coops remained positive, even after steam cleaning. The provision of clean transport coops has been recognized as one of the last steps in limiting external contamination of the birds prior to their entry into the processing plant. Typically, birds arriving at the plant have frequently been contaminated with *Salmonella* (60–100%) and *Campylobacter* (80–100%) (Wempe *et al.*, 1983; Acuff *et al.*, 1986; Kotula and Pandya, 1995). *Salmonellas* are known to attach firmly to the skin of live broilers (Lillard, 1989) and all indications are that avoidance of contamination is preferable to any attempt at remedial action. *Campylobacter* is even more widespread in poultry plants and the organism has been recovered from scald tanks, feathers, chill tanks and other processing equipment (Wempe *et al.*, 1983). Furthermore, shackles and tanks were contaminated with *Campylobacter* in broiler, layer, turkey and duck processing plants (Baker *et al.*, 1987).

13.2.3 Slaughter line

Killing stage

The blade of the neck-cutting knife was cultured for *Salmonella* contamination in a Spanish processing plant (Carramiñana *et al.*, 1997). In this study, 20 moistened swabs were used to sample the knife and 50% were found to be positive. Using a marker organism, an automatic killing knife was shown to spread the organism even to the 500th bird passing through the machine (Mead *et al.*, 1994), but the presence of chlorine in the spray-wash water directed at the knife reduced the spread of the marker by 50%. Although *Campylobacter* was not evaluated in this study, the known spread of both *E. coli* and *Salmonella* would suggest that the blade of the killing knife would be equally capable of spreading *Campylobacter* among the carcasses.

Scald tank

In some plants, the scald tank is considered to be the major site of cross-contamination with *Salmonella* (Mulder *et al.*, 1977) and *Campylobacter* (Wempe *et al.*, 1983). Normally, the scald tank reduces levels of pathogenic bacteria on carcasses, when operating properly. The greatest reductions in both

Salmonella and *Campylobacter* have been recorded at scald temperatures of 58–60 ºC, compared to 52 ºC (Notermans and Kampelmacher, 1975; Mulder *et al.*, 1977; Notermans *et al.*, 1977; Oosterom *et al.*, 1983; Wempe *et al.*, 1983). Unusual and unacceptable conditions include an inadequate input of clean water and high levels of organic material. Humphrey (1981) reported that salmonellas are more heat-resistant in the presence of organic matter, which can also reduce the pH value of the scald water.

The incidence of *Campylobacter* on feathers, skin and feet sampled prior to carcasses entering the scald tank ranged from 45 to 82.5%, with feathers, breast skin and foot pads being most often contaminated; overall levels of *Campylobacter* on carcasses ranged from 6.1 to 7.5 log cfu (Kotula and Pandya, 1995). Similarly, the incidence of *Salmonella* ranged from 27.5 to 75% before scalding and, again, feathers, breast skin and feet were most often contaminated. Numbers of *Salmonella* on carcasses pre-scald were 5.8–7.2 log units per carcass (Kotula and Pandya, 1995). The high incidence of both organisms prior to scalding suggests a CCP in relation to the spread of these pathogens. Scald tanks, when operated properly, give a 2–3 log reduction in levels of *Campylobacter* on the carcasses (Oosterom *et al.*, 1983; Izat *et al.*, 1988; Berrang *et al.*, 2000). Turkey carcasses have an incidence of *Campylobacter* contamination that is similar to broilers (Luechtefeld and Wang, 1981; Yusufu *et al.*, 1983; Dromigny *et al.*, 1985; Acuff *et al.*, 1986). Oosterom *et al.* (1983) reported that levels decreased on carcasses after scalding at temperatures of 50 ºC or more. Similar to observations in broiler processing plants, these levels tended to increase during removal of the feathers. On the other hand, on-line washing and exposure to chilling reduced *Campylobacter* to undetectable levels in some cases (Acuff *et al.*, 1986).

Importantly, chickens brought from farms where *Salmonella* was not detectable remained uncontaminated during processing, when slaughtered first in the day (Sarlin *et al.*, 1998). Thus, it is important to consider the linkage between contamination problems in the process and conditions on the farm. Problems in the processing plant could be largely avoided by effective *ante-mortem* intervention strategies.

Defeathering

Feather removal is consistently cited as a major point of cross-contamination for both *Salmonella* and *Campylobacter*. Bryan *et al.* (1968a) suggested that the microbial status of the carcass after defeathering is a good indicator of the likely condition of the final product. When pathogens are present, they are spread by aerosols and contaminate both carcasses and surrounding equipment in the defeathering area (Kotula and Kinner, 1964; Bryan *et al.*, 1968a; Patterson, 1973; Oosterom *et al.*, 1983; Georgnaras *et al.*, 1997). It was also observed that the action of the rubber fingers used in defeathering could drive the organisms into the skin tissue and feather follicles (Bryan *et al.*, 1968a).

The significance of defeathering as a potential source of cross-contamination is difficult to overstate. Indeed, the numbers of *Campylobacter* on carcasses

virtually doubled in some studies (Oosterom *et al.*, 1983; Wempe *et al.*, 1983; Yusufu *et al.*, 1983; Acuff *et al.*, 1986; Izat *et al.*, 1988; Berrang *et al.*, 2000). Similarly, contamination of skin and water with *Salmonella* and *Campylobacter* occurred as the feathers were removed (Wempe *et al.*, 1983; Yusufu *et al.*, 1983; Geornaras *et al.*, 1997). These data further emphasize the need to optimize conditions in the scald tank in order to reduce pathogens to a minimum prior to feather removal.

The hands of operatives in the defeathering area were found to have up to 5.26 log cfu of *Campylobacter* (Oosterom *et al.*, 1983). In an experimental study, the hands of volunteers were artificially contaminated with *Salmonella* (500–2,000 organisms) and then the fingertips sampled regularly. The *Salmonella* could be detected for up to three hours after inoculation (Pether and Gilbert, 1971). Mead *et al.* (1994), using a marker strain of *E coli* on several initial carcasses, found that the marker could be detected consistently on the 200th carcass passing through the defeathering machines. Furthermore, the marker organism could be detected on the 20th revolution of the conveyor belt from which carcasses were transferred from the killing line to the evisceration line. Similarly, when a fluorescent marker was placed in the crops of live birds prior to hanging on the processing line, more than 60% of subsequent carcasses were contaminated externally with the marker before being transferred to the evisceration line (Byrd *et al.*, 2002). These studies suggest that, even before evisceration, a majority of carcasses will already be contaminated with any pathogens present. This phenomenon may serve to reduce the effectiveness of intervention programs that are currently used in processing plants.

Evisceration and inspection

Defeathering and evisceration are frequently seen as areas that require specific attention, due to the high rate of cross-contamination (Saleha *et al.*, 1998). Workers, equipment and contaminated carcasses have been shown occasionally to cross-contaminate 'clean' carcasses (Stewart, 1965). The main focus of the cross-contamination problem has been the possible cutting and tearing of the viscera (Bryan and Doyle, 1995). More recent work has also focused on the leakage of crop contents onto the carcass during processing (Hargis *et al.*, 1995; Byrd *et al.*, 1998). Hargis *et al.* (1995) found that crops ruptured 86 times more frequently than ceca during processing and were far more likely to be *Salmonella*-positive (Hargis *et al.*, 1995; Corrier *et al.*, 1999a) and contaminated with *Campylobacter* (Byrd *et al.*, 1998).

While several studies have shown that the incidence of *Salmonella*-positive carcasses tends to increase during processing, up to the point of immersion chilling (James *et al.*, 1992; Carramiñana *et al.*, 1997; Sarlin *et al.*, 1998), both the levels and frequency of *Campylobacter* contamination have decreased pre-chilling in most processing plants investigated (Oosterom *et al*, 1983; Wempe *et al.*, 1983, Yusufu *et al.*, 1983; Izat *et al.*, 1988; Berrang and Dickens, 2000). This marked difference in behavior may be related to the ability of *Salmonella* to recognize and bind to specific receptors on carcass tissues and to adhere in areas

(e.g. cut surfaces) where they are relatively protected from washing or disinfection (Lillard, 1988). Less is known, however, about the attachment capabilities of *Campylobacter*.

Powell *et al.* (1995) examined inspection-passed and re-processed broiler carcasses with regard to *Salmonella* and *Campylobacter* contamination. In this study, randomly-selected carcasses that had passed the inspection stage had a 9% *Salmonella* contamination rate, while 26% sampled prior to washing or re-processing had visible fecal contamination. A similar batch of inspected carcasses had an 85% *Campylobacter* contamination rate in comparison with 95% of carcasses that were fecally contaminated prior to washing or re-processing. With respect to the proportions carrying *Campylobacter*, re-processed carcasses were not significantly different from those that had passed the inspection stage initially (84% v. 74%). Unfortunately, however, the present inspection system does not provide the inspector with any means of identifying carcasses that are contaminated with pathogens (Bryan and Doyle, 1995). In one study, the inspectors themselves may have contributed to *Campylobacter* contamination by handling the carcasses (Oosterom *et al.*, 1983).

Recently, a fluorescent marker was inoculated into the crops of broilers and followed through the processing plant (Byrd *et al.*, 2002). The presence of the marker was observed on the external surfaces of carcasses at an incidence of 67% at the manual or automatic re-hanging station, 82.5% post inspection, 92% pre crop-removal, 94% post crop-removal and at 53% after the final wash, prior to immersion chilling. These data suggest that contamination of carcasses with the contents of the upper gastrointestinal tract, known to frequently contain *Salmonella* and *Campylobacter*, is a relatively common occurrence, at least in some processing plants, regardless of the ability of inspectors to recognize visible ingesta. The high frequency of ingesta that is *invisible* to the naked eye is probably the reason why the presence of visible ingesta does not appear to be a good predictor for the presence of *Salmonella* on processed broilers (Young and co-workers, unpublished data).

Immersion chilling

The purpose of immersion chilling is to reduce carcass temperature. Since the carcasses enter one or more common tanks of cold water, the immersion process is clearly a potential source of cross-contamination. It is critical, therefore, that the required temperature, water usage, pH and free chlorine levels are maintained for effective control of pathogens (Bryan and Doyle, 1995). When chillers are operated properly, carcass-associated bacteria are partially removed and the overall microbiological quality of carcasses can actually be improved. On the other hand, poorly maintained chilling systems lead to cross-contamination of carcasses (Sarlin *et al.*, 1998).

Contamination of the end-product with pathogens varies widely between individual plants and among published investigations. For example, contamination rates for *Campylobacter* ranged from 64 to 99% with counts varying from < 1.0 to 4.19 log cfu per unit sample (McBride *et al.*, 1980;

Oosterom *et al.*, 1983; Wempe *et al.*, 1983; Yusufu *et al.*, 1983; Juven and Rogol, 1986; Izat *et al.*, 1988; Berndtson *et al.*, 1992; Waldroup *et al.*, 1992; Humphrey *et al.*, 1993; Cason *et al.*, 1997; Berrang and Dickens, 2000). Similarly, *Salmonella* recovery from post-chill carcasses has ranged from 0 to 97.3%, with generally lower counts than those of *Campylobacter* (Bryan *et al.*, 1968 a,b; Thiessen *et al.*, 1984; James *et al.*, 1992; Waldroup *et al.*, 1992; Carramiñana *et al.*, 1997; Cason *et al.*, 1997; Palmu and Camelin, 1997).

13.3 Improved technologies to prevent contamination

Salmonella and *Campylobacter* spp. originate in the digestive tract of the live bird and are spread by cross-contamination, which can occur at many points in rearing and processing where fecal matter is present. Changes in prevalence and numbers of these organisms during processing are shown in Tables 13.1 and 13.2. In the plant, much of the cross-contamination occurs during scalding, picking and chilling (Lillard, 1989). Reports indicate that 1–50% of carcasses become contaminated with pathogens, such as *Salmonella, Campylobacter, Listeria monocytogenes, Clostridium perfringens* and *Staphylococcus aureus*, during the normal course of processing (CDC, 1984). More recently, a report showed that 88.2% of commercial broilers tested positive for *C. jejuni*/*coli* (USDA, 1995). Furthermore, it has been estimated that between 20% (USDA, 1995) and 35% (Lillard, 1989) of ready-to-market broilers carried *Salmonella*, whereas only 3–4% of birds entering the plant were *Salmonella*-positive.

Because *Salmonella* and *Campylobacter* are present in the feces of carrier birds, carcass picking and evisceration can be major sites in processing where bacteria are transferred from the intestines to the skin. Scalding and chilling, too, are processing steps where cross-contamination from one carcass to another can occur; however, scalding usually decreases overall microbial levels on carcasses (Gardner and Golan, 1976), especially when multiple tanks are used in sequence. Picking and viscera removal are generally associated with increases in microbial contamination (Izat *et al.*, 1988), although modern evisceration systems that keep carcasses and viscera separate on two parallel processing lines represent an improvement in this stage of the process.

13.3.1 Management of immersion chillers

During processing, one of the most critical steps in controlling the growth of microorganisms is carcass chilling. Currently, two methods exist: counter-current immersion chilling and air chilling. Counter-current immersion chilling is applied universally in the USA. In this process, carcasses are transferred to a tank of water that is flowing in the opposite direction to the movement of the carcasses. A counter-current flow allows the carcasses to come into contact with the coldest, cleanest water at the exit end of the chiller. The rate of water overflow is specified for each carcass that enters the chiller to minimize the

Table 13.1 *Salmonella* prevalence and numbers in the processing plant[1,2]

Reference	Pre-scald	Post-scald	Post-picking	Evisceration	Pre-chill	Post-chill
Bryan *et al.* (1968a)[3]						11–21%
Bryan *et al.*(1968b)			75.6%	28.9%		
McBride *et al.*(1980)	38.8%			17.7%		21.5%
Thiessen *et al.*(1984)						97.3%
James *et al.*(1992)			58%		48%	72%
Waldroup *et al.*(1992)						25.9–77.3%
						(0.1–0.7)
Humphrey *et al.* (1993)		68.6%[4]				
		74%[5]				
Kotula and Pandya (1995)	28–7.5%					
	(5.8–7.5)					
Carramiñana *et al.* (1997)	50%	75%	55%	60%	70%	60%
Cason *et al.* (1997)			23%		20%	19%
Palmu and Camelin (1997)				50%		52%
Georgnaras *et al.* (1997)	100%	80%	100%			
Sarlin *et al.* (1998)			12%		89	68%
					39%	
					31%	

[1] Prevalence values represents *Salmonella* samples positive/total cultured (%).
[2] Numbers represent the number of *Salmonella* cells present (log cfu).
[3] Turkey samples
[4] *Salmonella* incidence taken from water pH 6.7
[5] *Salmonella* incidence taken from water pH 9.0

Table 13.2 *Campylobacter* prevalence and numbers in the processing plant[1,2]

Reference	Pre-scald	Post-scald	Post-picking	Evisceration	Pre-chill	Post-chill
Luechtefeld and Wang (1981)[3]					94%	34%
Wempe *et al.* (1983)	18.3% (3.86–4.89)	27.8% (<1.0–2.2)	94.4% (1.5–5.0)		80.6% (1.3–4.3)	80–100% (1.0–3.40)
Oosterom *et al.* (1983)	3.42	1.25	3.34	1.20–4.26	2.58	1.24
Yusufu *et al.* (1983)[3]	13.3% (1.5–3)	5.6% (1.3)	80.6% (1.0–4.64)		23–44.4% (1.46)	22.2% (1.03)
Acuff *et al.* (1986)[3,4]	50–100% 1.7–2.8	0–0.45	0.2–2.6	0.26–2.6	0.2–2.6	1–1.55
Izat *et al.* (1988)	<0.98–3.74	<1.26	2.37–3.68	2.02–3.49	1.71–3.04	1.18–1.85
Humphrey *et al.* (1993)		23.2–54.9%				
Kotula and Pandya (1995)	45–82.5% (6.1 to 7.5 cfu)					
Juven and Rogol (1986)					82.5%	77.5%
Cason *et al.* (1997)		63%			100%	99%
Berrang *et al.* (1999)	4.36 cfu	1.81cfu	3.62 cfu	3.05 cfu	2.46 cfu	0.99cfu

[1] Prevalence values represent *Campylobacter* samples positive/total cultured (%).
[2] Numbers represent the number of *Campylobacter* cells present (log cfu).
[3] Turkey samples
[4] MPN procedure

build up of microbes and suspended solids (Houston, 1985). In addition, 20–50 ppm of chlorine is allowed in the chill-water, as an aid to controlling microbial levels (USDA, 1973). The entire process is designed to reduce the carcass temperature so that the growth of microorganisms is inhibited. For broilers, the USDA requires that the carcass temperature is reduced to 4 ºC within four hours (USDA, 1973).

Because poultry carcasses are immersion-chilled in the USA, they retain a certain amount of water. Water retention and the fact that all carcasses come into contact with contaminated chill-water raises concerns about the potential for cross-contamination. Although, essentially, carcasses are being washed during immersion chilling, they are also being exposed to any pathogens present in the chill-water. Currently, the USDA requires poultry processors to indicate the presence of added water, a by-product of washing and chilling, on package labels (USDA, 2002).

The red-meat industry in the USA uses air chilling as a means of reducing carcass temperature without water uptake. In fact, incorporation of additional moisture is prohibited in the processing of beef, pork and lamb. Air chilling of carcasses is an alternative to water-immersion processes and is currently used in Europe for all meat processing, including poultry. The use of added chlorine as an antimicrobial agent in process water is prohibited in Europe; therefore, other means of controlling pathogens in processing are needed. For air chilling, carcasses are chilled on-line, so that, theoretically, there is less chance of microbial cross-contamination.

An early study on chilling methods indicated that spoilage occurred sooner with immersion-chilled broilers than those that were air-chilled (Knoop, 1971). In fact, psychrotrophs predominated on the carcasses after immersion chilling, ultimately leading to a more rapid rate of spoilage. Contrary to these findings, Thomson *et al.* (1975) reported that air-chilled carcasses had higher microbial counts during storage than those that were immersion-chilled. However, differences between the two studies could have been due to the relative cleanliness of the immersion chiller, increased handling of air-chilled carcasses and, in the second study, variables in the air-chilling processes used.

Studies have shown varying results when evaluating the role of the immersion-chiller as a source of carcass contamination. For example, James *et al.* (1992) reported an increase in the prevalence of *Salmonella* following immersion chilling, while Cason *et al.* (1997) found no change in *Salmonella* prevalence and a decrease in the prevalence of *Campylobacter*. Cason *et al.* (1997) reported contamination rates for *Salmonella* and *Campylobacter* of 20 and 94% respectively, post-chill. These differing levels may indicate why immersion chilling can be effective in reducing pathogens in some cases and not in others. Where the proportion of contaminated carcasses is high, as with *Campylobacter*, the physical process of washing the carcasses in the chiller may actually reduce levels on individual carcasses, without changing the overall prevalence. However, in situations where pathogen prevalence is lower (as with *Salmonella*), the likelihood of spreading the organism to a previously negative

carcass may actually be greater. In plants where *Campylobacter* levels on carcasses were high, Izat *et al.* (1988) reported that there was a reduction in numbers due to immersion chilling, but not in plants where *Campylobacter* levels were already low prior to chilling.

13.3.2 Controlling pathogens

To minimize microbial contamination of poultry, a number of physical and chemical treatments can be employed during processing. Mechanical processes, such as removal of feathers and viscera, and washing, reduce the microbial load of carcasses. In addition, combined treatments, such as heat and a chemical treatment, are typically used to further reduce microbial contamination of carcasses. The effectiveness of the various treatments depends on the numbers of bacteria present initially and the degree of their attachment to, or entrapment in, the skin. Bacterial attachment is considered to be a two-stage process in which the initial phase is reversible and depends on the retention of bacteria in a liquid film, and physical forces that may promote or inhibit bacterial movement and entrapment from this film (Dickson and Anderson, 1992). This initial phase is followed by an irreversible, second phase involving the formation of extracellular fibrils that aid attachment and involve production of polysaccharide. The mechanism of the second phase is complex, but the attachment is stronger than that of the initial phase. Early research showed that factors influencing bacterial attachment to poultry skin include pH, time, temperature and possession of functional flagella (Notermans and Kampelmacher, 1974). Bacteria with flagella were found to bind more readily to the carcass surface than those without. In addition, ambient temperature (20 ºC) and a slightly alkaline pH value (pH 8.3) were found to promote bacterial attachment. Therefore, the stage of attachment, as well as the physical and chemical environment, can influence bacterial attachment and the effectiveness of both physical and chemical treatments in removing bacteria from the carcass surface.

13.3.3 Spray-washing of carcasses

Inside/outside carcass washers are used in poultry processing to reduce visible, fecal contamination on carcasses and therefore microbial levels before the chilling stage. Spraying carcasses with water or an antimicrobial solution is a method commonly employed to reduce carcass contamination. Typically, the equipment consists of a stainless-steel cabinet containing a series of mounted spray-nozzles (Fig. 13.1). Generally, some spray-nozzles are positioned to spray the outside of the carcass, while others are required to wash the abdominal cavity. In some cases, spray washers may function as outside washers only, whereas others carry out both tasks within a single unit.

Researchers have shown that both treatment time and water pressure influence the efficacy of washers in reducing bacterial levels on carcasses. For example, Xiong *et al.* (1998) found that, when poultry carcasses were treated

Fig. 13.1 Inside/outside carcass spray-washer.

with a water spray at 207 kPa for 30 sec, a one-log reduction in *Salmonella* was achieved. Other researchers have shown that spray washing reduced *Salmonella* by 0.4 log when carcasses were sprayed at 413 kPA for 17 sec (Yang *et al.* 1998).

Poultry processors often use this step as a CCP in the HACCP system to meet USDA requirements for zero fecal contamination of carcasses. An optimum water pressure is needed to minimize the number of carcasses contaminated with feces or ingesta during slaughter. In a study by Brashears *et al.* (2001), it was found that a pressure of only 20 psi was not sufficient to remove all fecal matter and ingesta from poultry carcasses, but when the pressure was increased to 42 psi, levels of generic *E. coli* on the carcasses actually increased. In further investigations, it was found that the optimum pressure was 30 psi and this reduced visible fecal material and ingesta, and also generic *E. coli.* It was postulated that the higher pressure may force bacteria into the skin and also promote cross-contamination. Therefore, water pressure needs to be optimized if it is to be an effective means of reducing carcass contamination. Other means of enhancing the effect of spray washing include the addition of antimicrobials and manipulation of water temperature.

Increasing the temperature of the water used in spray washers was found to be an effective means of reducing carcass contamination. Also, steam has been studied as a surface decontamination step (Morgan *et al.*, 1996). While steam pasteurization has been effective in small-scale trials, it is difficult to apply evenly to individual carcasses, especially when carcasses are moving at line-speeds up to 140 per min. There is the additional possibility that steam pasteurization may 'cook' the skin, if not applied correctly.

13.3.4 Use of super-chlorination

One of the most common chemical treatments used in poultry processing is super-chlorination of process water. Chlorinated water has been used in immersion chillers for many years and is also applied in spray washing. Commercially, chlorine is used mainly in the form of hypochlorite, which is more convenient than, for instance, chlorine gas. With the use of sodium or calcium hypochlorite in water treatment systems and as disinfectants, chlorine and chlorine-based compounds have gained widespread acceptance as effective sanitizers. (*Note:* chlorine and hypochlorite will be used here as interchangeable terms; however, the most prevalent form of chlorine for food-industry use is hypochlorite.) Chemically, chlorine is an oxidizing agent. Tap water is usually chlorinated at a concentration of 1–3 ppm. Liquid bleach, such as Clorox^{TM}, is sodium hypochlorite in water. Household bleach in liquid form contains approximately 5.25% sodium hypochlorite. When chlorine is added to water, the active antimicrobial formed is hypochlorous acid (HOCl): $Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$. In the presence of large concentrations of organic matter, free chlorine is either inactivated or it may persist in combined form as chloramines, which are less effective as antimicrobials.

As a cleaning agent, chlorine is very effective in removing protein residues and, to a lesser extent, carbohydrate material from contaminated surfaces. Cleaning with chlorine removes visible soil and/or food particles from processing equipment and physically reduces the microbial load. The efficacy of chlorine as a bactericidal agent is affected by the pH value of the solution and the amount of organic material present. In practice, the efficacy of chlorine in reducing bacterial levels decreases with increasing pH and increasing organic load. During cleaning, organic loads are usually high and alkaline detergents are often used; therefore, chlorine has little antimicrobial activity under these circumstances, and there is little or no hypochlorous acid formed at pH 7.0 or above.

In poultry chillers, the chlorine is normally added as hypochlorite. Levels of free available chlorine range from a minimum of 20 ppm to 50 ppm, the maximum allowed. Because the chiller environment has a relatively high organic load, the effectiveness of chlorine is often reduced. Also, the pH of the chill-water needs to be slightly acidic, so that the most active form of chlorine, hypochlorous acid, is present. The pH values most conducive to the formation of hypochlorous acid are in the pH range 4.0–6.0.

Chlorine was first used in US poultry processing plants in the 1950s to extend product shelf-life. However, it appears to have little direct effect on carcass bacteria and Lillard (1993) showed that attached or entrapped salmonellas are not readily accessible to chlorine and therefore are relatively unaffected by it. Even the more sensitive *C. jejuni* is almost unharmed when attached to chicken skin (Mead, unpublished data). The main benefit from chlorination of process water lies in its ability to control microbial contamination of the processing environment and equipment. Bailey *et al.* (1986) showed that a chlorine concentration of at least 40 ppm was required for continuous disinfection of eviscerating equipment via chlorinated water sprays. In relation to water immersion chilling, Mead and Thomas (1973) showed that chlorine at a total residual of 45–50 ppm could be used to keep the chill-water virtually free from viable bacteria, thereby reducing the opportunity for cross-contamination of carcasses.

13.3.5 Chlorine dioxide

Another chlorinated compound used to disinfect public water supplies that is finding application in the food industry is chlorine dioxide (ClO_2). The gas is soluble in water, does not react with ammonia or nitrogenous compounds, as chlorine does, has a greater oxidizing capacity than chlorine and its lethal effect on bacteria is not affected by high pH. Chlorine dioxide is generated by mixing sodium chlorite with chlorine gas, as described in eqn 13.1 or by mixing sodium hypochlorite with hydrochloric acid (eqn 13.2):

$$Cl_2 + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl \qquad 13.1$$

$$HCl + NaOCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + NaOH \qquad 13.2$$

Lillard (1979) compared the effectiveness of chlorine and ClO_2, when added to the water in immersion chillers. Use of ClO_2 was found to be seven times more effective than adding chlorine and this was attributed to the greater solubility and oxidizing capacity of ClO_2, as well as a lower reactivity with organic matter. In consequence, ClO_2 could be used at relatively low concentrations (3–5 ppm), providing residuals of about 0.2–0.5 ppm. Another study (Villarreal *et al.*, 1990), showed that chilling carcasses in an ice-water mixture containing 1% of a slow-release ClO_2 product (Alcide Corp.) reduced *Salmonella* contamination to undetectable levels.

13.3.6 Other chemical treatments

In addition to the use of chlorine and ClO_2, many attempts have been made to develop chemical decontamination treatments. Examples of those studied, and even used in commercial processing, are described below. Some have been approved by USDA for use in automated re-processing of fecally contaminated carcasses. Ideally, a treatment should:

- have no effect on product appearance, smell, taste or nutritional properties
- leave no residues and therefore pose no threat to the environment

- encounter no objections from consumers or legislators
- be cheap and easy to apply
- improve shelf-life by inactivating spoilage organisms, as well as pathogens
- be compatible with modified atmosphere packaging (Hinton and Corry, 1999).

Sanova

This product is manufactured by the Alcide Corp. and is an acidified form of sodium chlorite. It is used at concentrations of 500–1200 ppm, either singly or in combination with various organic acids, to achieve pH values of 2.3–2.9 for automated carcass re-processing. Lower concentrations (50–150 ppm) are used in immersion chillers. Kemp *et al.* (2001) showed that Sanova reduced the prevalence of *Salmonella* on poultry carcasses from 31.6% to 10%.

Trisodium phosphate

Trisodium phosphate (TSP) is an alkaline detergent that has been approved by USDA for use as a spray-application or carcass-dip at concentrations of 8–12% (21 CFR 182.1778). The treatment temperature should be maintained at 45–55 ºF to ensure efficacy. As an antimicrobial, TSP removes attached bacteria from carcass surfaces by means of its surfactant properties and high alkalinity (pH about 12.0). In addition, TSP kills bacteria by disrupting the cell membrane and causing leakage of cellular material (Giese, 1993). Slavik *et al.* (1994) found that *Campylobacter* levels were significantly reduced when poultry carcasses were dipped in a 10% TSP solution after chilling. In another study, TSP (8%) was shown to be an effective means of reducing levels of *S*, Typhimurium on chicken skin (Tamblyn *et al.*, 1997). A 1.48 log-reduction was reported when the bacterial cells were loosely attached and a 1.60 log-reduction when attachment was firmer. Spray pressure and application time have been shown to influence the efficacy of TSP treatment. Li *et al.* (1997) determined the efficacy of spray application, using a 10% TSP solution at pressures of 207, 345 and 827 pKa, respectively. It was concluded that a pressure of 827 pKa and a contact time of 30 sec were most effective and reduced *S.* Typhimurium by 1.82 log units per carcass.

Although, TSP is effective in reducing microbial counts from poultry, there are some concerns about using the compound. In particular, the high pH can cause problems when the spray-application method is used prior to carcasses entering the chiller. The resultant increase in the alkalinity of the chill-water to pH 9.5–10.5 decreases the antimicrobial activity of added chlorine. Following treatment, any attempt to remove residual TSP by spray-washing the carcasses would only add to treatment cost and complexity. In addition, large amounts of phosphate in the wastewater represent an environmental issue. Finally, like many chemical treatments, the compatibility of phosphate compounds with processing equipment must be considered. Over time, phosphates can be corrosive to metal equipment, although less so than substances such as chlorine.

Lactic acid

Among the naturally occurring organic acids that could be used for decontamination purposes, lactic acid has been favored because of its traditional involvement in controlling the spoilage of fermented foods and the lack of adverse effects, at appropriate concentrations, on the sensory properties of poultry carcasses. Applications to raw meat and poultry have been reviewed by Smulders (1987). Lactic acid treatment of broiler carcasses by immersion in a 1–2% solution reduced microbial contamination by about one log unit per gram of skin (van der Marel *et al.*, 1988). The acid is most active in this respect in the undissociated state and it has both bacteriostatic and bactericidal properties. Part of its action depends on the fall in pH that occurs. The acid has a lethal effect on *Salmonella, Campylobacter* and other Gram-negative pathogens and shows a delayed bacteriostatic effect during storage of treated meats (Smulders, 1987). Also, the shelf-life of the product is extended. Since the bactericidal effect is increased at 35 ºC, it could be beneficial to treat carcasses with a warm solution early on in the process and before microbial contaminants become firmly attached to carcass surfaces. While spray application is recommended, immersion treatment may be preferable to ensure that the lactic acid comes into contact with all parts of the carcass.

Because concentrations of lactic acid above 1.5% may cause discoloration of the carcass, Zeitoun *et al.* (1999) have preferred the use of a 10% lactic acid/sodium lactate buffer (pH 3.0), which retains a decontaminating effect, but avoids any sensory problems. This treatment was used in conjunction with modified atmosphere packaging to prolong product shelf-life.

13.4 Acid treatment of ready-to-eat products

Acid marinades are becoming more popular as antimicrobial treatments, particularly for their ability to reduce *L. monocytogenes*, when incorporated in ready-to-eat meat products. Typical marinades that are utilized for their antimicrobial properties include sodium and potassium lactate, sodium citrate, sodium lactate combined with sodium diacetate and combinations of sodium lactate with potassium lactate and diacetate. Sodium lactate levels permitted in the USA for cooked poultry products are 2.9% pure sodium lactate or 4.8%, when using a 60% lactate solution. In formulating products that include sodium lactate, the salt concentration would probably need to be reduced, as the sodium lactate enhances the salty flavor. According to US regulations, sodium acetate and diacetate are approved as flavoring compounds at a maximum level of 0.25% of the formulation weight (Keeton, 2001). Research has shown that sodium lactate in cooked, strained beef and beef roasts, and sodium diacetate in turkey slurry reduced the growth of *L. monocytogenes* (Schlyter *et al.*, 1993; Miller and Acuff, 1994). However, during refrigeration, surviving cells increased in number. When cooked chicken was treated with lactate and dipped in a *L. monocytogenes* cocktail, the organisms were shown to have a longer lag

phase than usual. Nevertheless, growth still occurred during refrigerated storage (Barakat and Harris, 1999). In general, it is thought that the main effect of sodium lactate and diacetate in inhibiting bacterial growth is by extending the lag phase.

While acid marinades may act as antimicrobials, they also have an impact on meat quality and functionality. Traditionally, acid marinades were used to improve the flavor and texture of prepared meats during storage. Where alkaline salt-phosphate brine systems serve to increase water holding capacity (WHC) and tenderize meat, marinades that are highly acidic (pH below 5.0) tenderize the meat by denaturing proteins, but do not improve WHC to the extent seen with alkaline brine. Mostly, salt and acid phosphates are used in combination with acid marinades to improve marinade retention. Other meat quality characteristics have been examined for stored products formulated with acid marinades. In this respect, Yang and Chen (1993) studied chicken fillets marinated in solutions of citric acid and Na_3PO_4 and found that Hunter Lab 'L' and 'b' values decreased ($p < 0.05$) while 'a' values increased ($p < 0.05$) with increasing storage time. It was postulated that the decrease in L value was related to the pH of the raw product. In another study, cooked, cured ham products were formulated with varying levels of sodium lactate, sodium diacetate or buffered sodium citrate. In comparing the different ham formulations for appearance, internal color, structure and firmness, only minor differences were observed between them. However, the addition of 0.2% sodium diacetate had a negative effect on product odor and taste (Stekelenburg and Kant-Muermans, 2003).

13.5 Future trends

Reducing microbial contamination of carcasses through the application of various intervention technologies and through disinfectants applied post-slaughter has been a common approach to preventing the spread of potentially pathogenic bacteria. Chemical treatments that should be investigated in the future include new packaging systems, such as edible films containing antimicrobials, that could be used to extend product shelf-life and decrease levels of foodborne pathogens. Alternatively, suitable antimicrobials could be incorporated in the normal types of packaging material to be released gradually. Also, the use of bacteriophage or bacteriocins that have bacteriostatic or bactericidal effects on target organisms could be included in routine, post-harvest intervention strategies. An example is nisin, a bacteriocin that is considered to be non-toxic and has received GRAS status in the USA. It has also been approved by the World Health Organization as a food preservative. Nisin inhibits microbial growth in milk, cheese, yogurt, eggs, vegetables and canned soups (Tu and Mustapha, 2002). Identifying suitable bactericidal compounds is essential to facilitate the control of foodborne pathogens in the poultry and meat industries.

Feed withdrawal, transportation, defeathering and evisceration are hazardous strategies in production that must be addressed in order to control the spread of

Salmonella and *Campylobacter*. The logical approach to controlling these pathogens would be to reduce their prevalence and numbers in birds entering the plant, together with continued intervention efforts during processing. A combined approach of this kind must be employed for cost-effective improvement in the microbial status of processed poultry. Commercial poultry companies vary widely with regard to the impact of individual control points. For effective intervention, it is essential that CCPs are confirmed for each stage in the production process. Indeed, the small cost of the exercise, in comparison with the relatively high cost of some available interventions, clearly warrants such an approach prior to the installation of any expensive equipment or the establishment of control procedures.

13.6 References

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14

Refrigeration and the safety of poultry meat

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14.1 Introduction

Slaughter, plucking and evisceration of birds results in a carcass with a deep temperature of approximately 40 °C and a surface with a temperature and a_w which are very conducive to the growth of pathogenic and meat-spoilage organisms. The main purpose of refrigeration is to reduce and then maintain the temperature of the meat below a value that will ensure a high-quality, safe product.

The refrigerated cold chain from slaughter to final consumption contains a number of operations designed to reduce the carcass temperature, i.e. primary and secondary chilling, and, where appropriate, freezing. It also contains operations, such as chilled and frozen storage, transport, retail display and consumer storage, that are designed to maintain, not change, the temperature of the meat. In addition, producers of poultry products are increasingly using frozen chicken in the form of carcasses, portions or boned-out blocks of meat as a raw material. This material has to be thawed, i.e. be above 0 °C, with no remaining ice, or tempered to between −5 and −2 °C for further processing. Frozen poultry is also commonly thawed before cooking or further preparation in catering operations and in the home.

14.2 Effects of low temperature on microbial survival and growth

A number of bacterial pathogens capable of causing food poisoning in humans are known to contaminate poultry meat. The most important are *Salmonella*, *Campylobacter* spp., *Clostridium perfringens*, *Listeria monocytogenes* and

Table 14.1 Minimum and optimum growth temperatures for pathogens associated with poultry meat (source: ICMSF, 1996)

	Minimum temperature (ºC)	Optimum temperature (ºC)
Campylobacter jejuni	32	42–43
Clostridium perfringens	12	43–47
Intestinally pathogenic		
Escherichia coli	c. 7–8	35–40
Salmonella	5.2*	35–43
Listeria monocytogenes	−0.4	37
Yersinia enterocolitica	−1.3	25–37

* Most serotypes fail to grow at < 7 ºC

enterohaemorrhagic *Escherichia coli* (Mead, 2004). In addition, Mead (2004) states that two cold-tolerant types of bacteria, *Aeromonas* spp. and *Yersinia enterocolitica,* are also found on poultry carcasses, but have not been associated with foodborne illness.

Minimum and optimum growth temperatures for pathogens commonly associated with poultry meat are shown in Table 14.1. Some pathogens, such as *L. monocytogenes*, are capable of growth at chill temperatures below 5 ºC. These are often cited as being of particular concern in relation to refrigerated meats, since refrigeration cannot be relied upon to prevent growth (Doyle, 1987). On the other hand, psychrotrophic pathogens are not particularly heat-resistant and adequate cooking should be sufficient to destroy them. Illness caused by *L. monocytogenes* has been associated with contamination of cooked, ready-to-eat products.

Microorganisms are broadly classified into three arbitrary groups (psychrophiles, mesophiles and thermophiles), according to the range of temperatures within which they may grow. A reduction in temperature below the optimum causes an increase in generation time, i.e. the time required for a doubling in number. It is an accepted, but crude, approximation that bacterial growth rates will double with every 10 ºC rise in temperature (Gill, 1986). Below 10 ºC, however, this effect is more pronounced and chilled storage-life is halved for each 2–3 ºC rise in temperature. Thus, the generation time for a pseudomonad (a common type of spoilage organism) might be one hour at 20 ºC, 2.5 hours at 10 ºC, 5 hours at 5 ºC, 8 hours at 2 ºC or 11 hours at 0 ºC. In the usual temperature-range for chilled meat, −1.5 to 5 ºC, there can be as much as an eight-fold increase in growth-rate between the lower and upper temperatures. Storage of chilled meat at -1.5 ± 0.5 ºC would achieve the maximum storage-life without any surface freezing.

The range of microorganisms capable of causing food spoilage is large; however, depending on the initial microflora and the growth environment, only a few species are generally found at spoilage. For example, under aerobic conditions, the predominant organisms on poultry are usually species of *Pseudo-*

monas, *Acinetobacter*, Psychrobacter and *Moraxella* (Mead, 2004). These organisms are psychrotrophs, i.e. capable of growth close to 0 ºC, but with much higher temperature optima. Only a small proportion of the initial microflora on meat will be psychrotrophic; the majority of organisms present are incapable of growth at low temperatures. As storage temperature rises, the number of species capable of growth will increase.

14.3 Refrigeration mechanisms and technologies

Heat can be lost from the surface of poultry by four basic mechanisms: radiation, conduction, convection or evaporative cooling. To determine the rate of heat transfer (Q_r) by radiation from poultry, the following approximation may be applied:

$$Q_r = eA\sigma\left(T_s^4 - T_e^4\right) \qquad 14.1$$

where e is the emissivity and A is the surface area of the food, σ the Stefan-Boltzman constant and T_s and T_e are the temperatures of the surface and the enclosure respectively.

To achieve substantial rates of heat loss by radiation, large temperature differences are required between the surface of the product and that of the enclosure. Such differences are not normally present during poultry cooling operations, except in the initial chilling of cooked products, i.e. rotisserie birds, chicken pies, etc. In industrial processing, the product to be cooled is often surrounded by other cooling products. This substantially reduces the opportunity for radiant heat exchange with the surfaces of the cooled enclosure. Heat gains due to radiation can be a problem in retail display of poultry.

The rate of one-dimensional heat transfer (Q_{cd}) by conduction is given by:

$$Q_{cd} = kA\frac{\partial T}{\partial x} \qquad 14.2$$

where k is the thermal conductivity of the medium through which the heat is passing and $\partial T/\partial x$ is the temperature gradient.

Physical contact between the product and the source of refrigeration is required to extract heat by conduction. The irregular shape of most poultry and poultry products precludes this mechanism in many applications. Plate conduction coolers are used for quick cooling of some packaged products and highly perishable products, such as mechanically-recovered or hot-boned meat. In these cases, they usually form the first stage of a freezing operation. However, the rate at which heat can be conducted away from the surface is not the sole criterion that governs the time taken to cool a product. Heat must also be conducted from within the product to its surface, before it can be removed. Most foodstuffs are poor conductors of heat and this imposes a severe limitation on attainable chilling times for either large individual items, i.e. poultry carcasses or small items, such as portions, cooled in bulk.

The rate of heat transfer Q_e from the surface of a food by evaporation/condensation is described by the equation:

$$Q_e = mLA(P_s a_w - P_m) \quad 14.3$$

where m is the mass transfer coefficient, A is the area, P_s is the saturated vapour pressure at the surface, a_w is the water activity, P_m is the vapour pressure above the food surface and L is the appropriate latent heat.

For most types of meat, the heat lost through evaporation of water from the surface is a minor component of the total heat loss. In a number of specific cases, however, such as the evaporative or spray cooling of poultry, it can be appreciable.

Most poultry refrigeration systems rely on convection as the principal means of heat removal. The rate of one-dimensional heat transfer (Q_c) by convection is given by:

$$Q_c = h_c A(T_s - T_a) \quad 14.4$$

where h_c is the convection or film heat-transfer coefficient and T_a the temperature of the cooling medium. The most common media are air and water, although salt solutions, sugar brines and other refrigerants have been used. Each combination of product and cooling system can be characterised by a specific surface heat-transfer coefficient. The value of the coefficient depends on the shape and surface roughness of the foodstuff and, to a much greater degree, on the thermophysical properties and velocity of the medium.

Mechanical refrigeration systems operate using the same basic refrigeration cycle (Fig. 14.1). A low-pressure, liquid refrigerant is allowed to evaporate to a gas within a coil. This process requires heat, which is extracted, thus cooling any medium surrounding the 'evaporator' coil. The gas from the evaporator is compressed in the 'compressor' to a high-pressure, hot gas. This gas is then passed through another coil, where it condenses and releases heat into any medium surrounding the 'condenser' coil. This high-pressure, cold, liquid refrigerant then passes though the 'expansion valve' back to the evaporator.

In a direct expansion system, the evaporator coil is either in contact with the meat to be refrigerated or the medium surrounding the meat, e.g. air, brine. In a secondary refrigeration system, a liquid, such as water or brine, is cooled by passing it over the evaporator coil. This cooled liquid can be used directly to cool the meat or be pumped through cooling coils in different parts of the plant, where it is used to cool air that is subsequently used to cool the meat.

14.3.1 Chilling

Air, spray/evaporative and immersion systems are the three most common methods of chilling dressed chickens. In the case of turkeys, which are usually larger, mechanical chilling in an ice and water mixture, or simple water immersion, are common methods, either as a first stage or a complete chilling process.

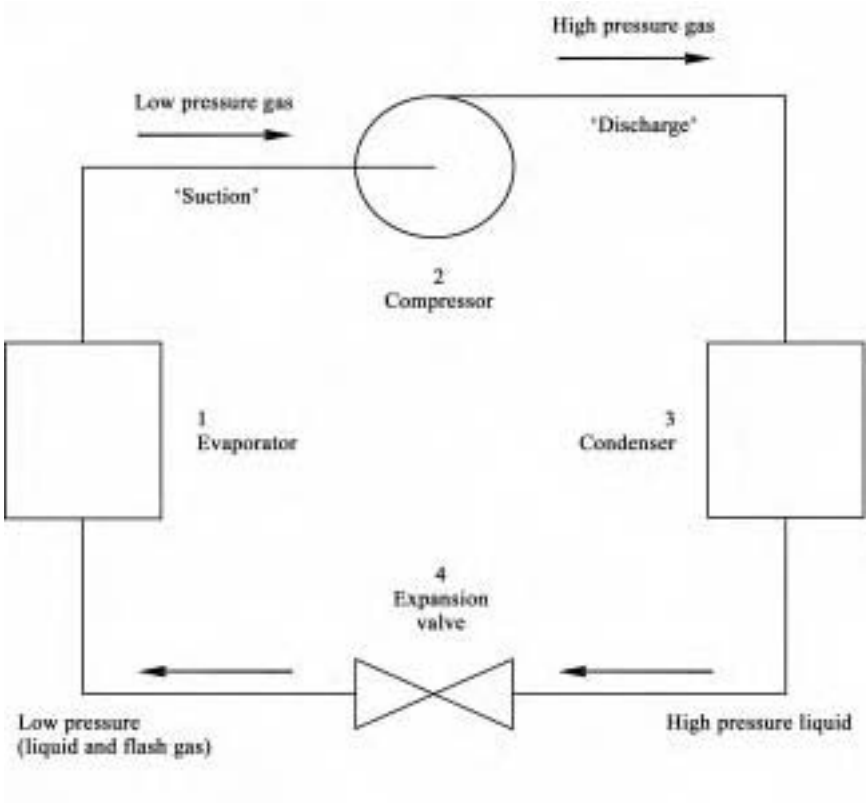

Fig. 14.1 Basic refrigeration system.

Air

Much of the poultry destined to be sold in an unfrozen state will be chilled in large, insulated rooms. Due to the scale of production in most poultry slaughterhouses, the dressed carcasses are normally conveyed on rails through the room or tunnel. In large plants, the birds will be transferred automatically from the evisceration line to the chill-room system. In other plants, carcasses are hung manually on long rails that extend through the chiller. Chilling time and weight loss will be a function of the environmental conditions within the chiller and the spacing between carcasses.

As the required throughput increases, there is a trend towards much larger chill rooms and more free space above and below the rails. This makes it easier to clean the chill room and maintain the rail system.

Spray/evaporative chilling

With spray-chilling systems that are used in conjunction with a soft-scalding process, the carcasses are transferred to an air chiller and sprays of potable water applied periodically, for example, at five and fifteen minutes after the start of

chilling, on four or five occasions during the whole chilling process. The point of the process is to increase the rate of evaporative heat loss and, by replacing the water that evaporates, reduce the overall weight loss from the carcass.

Immersion
Most poultry that is destined to be frozen is chilled initially by being immersed in chilled water or ice and water mixtures. The process used in Europe is normally a counter-flow system, in which the birds are conveyed in the opposite direction to the water flow, to minimise cross-contamination. The carcasses are either pulled through a large trough by a moving-rail system, or chilled in a mechanical system, where an Archimedes screw is used to move the carcasses through a counter-current ice and water mixture.

14.3.2 Freezing

Freezing of poultry in the form of carcasses, portions or boned-out pieces is normally carried out in an air-blast freezer; however, plate freezers are often used for offal and low-value materials.

Blast air
Systems range from the most basic, in which a fan draws air through a refrigerated coil and blows the cooled air around an insulated room, to a purpose-built, blast-freezing tunnel fed by a conveyor. The big advantage of air systems is their versatility, especially when there is a requirement to freeze a variety of irregularly-shaped products.

Poultry meat is usually boxed and frozen on racks or trolleys. Individual portions may be frozen in tunnel or spiral freezers and packaged after freezing. Freezing times for cartons of poultry carcasses or joints are long, typically more than ten hours. Continuous freezing equipment is therefore used only in very high throughput plants.

Plate freezing
Contact refrigeration methods are based on heat transfer by contact between products and metal surfaces, which, in turn, are cooled by either primary or secondary refrigerants. Contact freezing offers several advantages over air-cooling, including much better heat transfer and significant energy savings. However, the need for regularly-shaped products with large flat surfaces is a major drawback.

14.4 The cold chain

There are few studies on changes in microbial contamination of poultry over the entire cold chain. However, Reddy *et al*. (1978) found that microbial numbers on turkey carcasses were reduced after every stage that they examined in a

Table 14.2 Microbial counts during the chilling, freezing and frozen storage of turkeys (Reddy *et al.*, 1978)

	Log no. bacteria cm^{-2}		
Stage	Mesophiles	Psychrotrophs	Coliforms
After evisceration	4.16^{a}	2.77^{a}	1.72^{a}
After spin chilling	2.75^{b}	2.15^{b}	1.57^{a}
After crust freezing	2.57^{bc}	1.60^{c}	0.93^{c}
Blast frozen	2.27^{c}	1.01^{d}	0.44^{c}
From holding freezer	2.21^{c}	0.86^{d}	0.08^{d}

Values in same column with different superscripts are significantly different ($p < 0.05$).

typical, commercial processing operation (Table 14.2). The key stages in the particular operation investigated were:

- spin chilling for approximately one hour
- crust-freezing in a brine immersion tank at −19 ºC
- blast freezing in air at −29 ºC
- holding in frozen storage at −21 ºC.

14.4.1 Chilling

Poultry are usually chilled in the form of whole carcasses and many studies have examined the effects of different chilling systems on the survival of both pathogenic and food-spoilage organisms.

Pathogenic organisms

Most studies on the effect of chilling on *Salmonella* contamination have used swabbing or whole-carcass rinses to determine the prevalence of positive carcasses before and after chilling, rather than the numbers of cells present (Table 14.3). Overall, the prevalence tends to be less after chilling. However, Morris and Wells (1970) noticed an increase when carcasses were chilled by rotation in an ice-slush and they believed that this was due to extensive contact between carcasses. Similarly, James *et al.* (1992a,b) found increases in *Salmonella*-positive carcasses when an immersion chiller was used without added chlorine, and no significant change with chlorine. In the case of *Campylobacter*, reductions of up to 2 log cfu per ml of rinse were obtained for carcasses that were water chilled (Table 14.4), but there was no change in an air-chilling system.

Water chilling also reduces the number of coliforms present on carcasses (Table 14.5). Reductions of 1.1 log cfu per ml of rinse were achieved without the use of chlorine and up to 2.5 log cfu per ml of rinse with chlorine addition. No significant reductions were achieved in air chilling. Similar results were obtained for *E.coli* (Table 14.6).

Table 14.3 Data on the effects on *Salmonella* of chilling poultry carcasses

Chilling method	Details	Sampling method	Measurement	Before chilling	After chilling	References
	Rotation in an ice slush	Swab samples from carcasses	% prevalence	4	11	Morris and Wells (1970)
Immersion chilling	At least 4 ºC after 1 hour in the chiller	Whole-carcass rinse	% prevalence	48	72	James *et al*. (1992a)
Immersion chilling	At least 4 ºC after 1 hour in the chiller, with chlorinated water (25 ppm)	Whole-carcass rinse	% prevalence	43	46	James *et al*. (1992b)
Immersion chilling		Whole-carcass rinse	% positive	20	19	Cason *et al*. (1997)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	1.3	0.8	Northcutt *et al*. (2003)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	% positive	56	15	Northcutt *et al*. (2003)
Air chilling	Near 3 ºC	Swab samples	% prevalence	70	60	Carramiñana *et al.* (1997)
Spin chilling		Swab samples from skin surface and body cavity of whole bird	% positive	52	13	Mikolajczyk and Radkowski (2001)

Table 14.4 Data on the effects on *Campylobacter* spp. of chilling poultry carcasses

Chilling method	Details	Sampling method	Measurement	Before chilling	After chilling	References
Immersion chilling		Whole-carcass rinse	% positive	100	99	Cason *et al.* (1997)
Immersion chilling		Whole-carcass rinse	Log cfu per carcass	5.31	3.80	Cason *et al.* (1997)
Immersion chilling		Whole-carcass rinse	Log cfu per carcass	5.39	3.91	Cason *et al.* (1997)
Water chilling	30 min at 1 ºC	Whole-carcass rinse	Log cfu per ml of rinse	2.1	0.7	Dickens *et al.* (2000)
Water chilling	30 min at 1 ºC, 2% solution of Protecta II	Whole-carcass rinse	Log cfu per ml of rinse	2.1	0.01	Dickens *et al.* (2000)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	2.9	1.6	Northcutt *et al.* (2003)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	% positive carcasses	99	83	Northcutt *et al.* (2003)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	5.5	5.3	Abu-Ruwaida *et al.* (1994)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	4.7	4.6	Abu-Ruwaida *et al.* (1994)

Table 14.5 Data on the effects on coliforms of chilling poultry carcasses

Chilling method	Details	Sampling method	Measurement	Before chilling	After chilling	References
Water chilling	30 min at 1 ºC	Whole-carcass rinse	Log cfu per ml of rinse	2.5	1.4	Dickens *et al.* (2000)
Water chilling	30 min at 1 ºC, 2% solution of Protecta II	Whole-carcass rinse	Log cfu per ml of rinse	2.5	0.04	Dickens *et al.* (2000)
Immersion chilling	25 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	3.36	2.65	Jimenez *et al.* (2002)
Immersion chilling	40 min at 5 ºC, 45 ppm chlorinated water	Body-cavity rinse	Log cfu per ml of rinse		1.28 reduction	Allen *et al*. (2000)
Immersion chilling	40 min at 5 ºC, 45 ppm chlorinated water	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin		1.10 reduction	Allen *et al*. (2000)
Immersion chilling	25 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	3.91	2.68	Jimenez *et al.* (2002)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	3.9	2.6	Northcutt *et al.* (2003)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	5.1	4.3	Abu-Ruwaida *et al.* (1994)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	5.2	5.0	Abu-Ruwaida *et al.* (1994)
Air chilling		Whole-carcass rinse	Log cfu per ml of rinse	3.27	2.59	Fluckey *et al.* (2003)

Table 14.6 Data on the effects on *Escherichia coli* of chilling poultry carcass cooling

Chilling method	Details	Sampling method	Measurement	Before chilling	After chilling	References
Immersion chilling	At least 4 ºC after 1 hour	Whole-carcass rinse	Log cfu per carcass	1.46	0.87	James *et al.* (1992a)
Immersion chilling	At least 4 ºC after 1 hour, chlorinated water (25 ppm)	Whole-carcass rinse	Log cfu per carcass	2.04	1.20	James *et al.* (1992b)
Immersion chilling	25 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	3.44	2.28	Jimenez *et al.* (2002)
Immersion chilling	25 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	2.49	1.60	Jimenez *et al.* (2002)
Water chilling	30 min at 1 ºC	Whole-carcass rinse	Log cfu per ml of rinse	2.0	0.9	Dickens *et al.* (2000)
Water chilling	30 min at 1 ºC, 2% solution of Protecta II	Whole-carcass rinse	Log cfu per ml of rinse	2.0	0	Dickens *et al.* (2000)
Immersion chilling	20 ppm chlorinated water	Whole-carcass rinse	Log cfu per ml of rinse	3.2	1.8	Northcutt *et al.* (2003)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	4.8	4.4	Abu-Ruwaida *et al.* (1994)
Air chilling	75 min at 0–5 ºC	Neck-skin 'maceration'	Mean log cfu per g of carcass neck-skin	4.2	4.1	Abu-Ruwaida *et al.* (1994)
Air chilling		Whole-carcass rinse	Log cfu per ml of rinse	3.08	2.2	Fluckey *et al.* (2003)

Total viable counts and spoilage organisms

Many studies have been carried out on the effects of chilling on microbial contamination of carcasses. In general, studies show either no change or a reduction in total viable counts (TVC) of Enterobacteriaceae. Some differences are apparent between the three main chilling methods.

Overall, immersion chilling, using water or ice-water mixtures, tends to produce a larger reduction in microbial contamination than either air- or spray-chilling methods. For example, Thomson *et al*. (1975) found that TVC from the breast skin of chicken carcasses decreased from an average of 3.1 log/cm^2 before chilling to 2.5 log after immersion chilling in tap water, followed by slush-ice, 2.7 log after air-blast chilling at −7 ºC, 3.5 m/sec. and 3.1 log after crust-freezing in air at −40 ºC. Cox *et al.* (1975) concluded that continuous immersion chilling in slush-ice results in a significant reduction in both TVC and counts of Enterobacteriaceae from broiler carcasses. Cason *et al.* (1997) observed that washing and immersion chilling of carcasses reduced TVC by 1.8 log per carcass and *Campylobacter* counts by 1.5 log.

Super-chlorination of water used in immersion chillers, a practice now illegal in the EU, tends to increase the washing effect of these processes. James *et al*., (1992b) reported that immersion chilling without chlorinated water seemed to have no effect on Enterobacteriaceae, but, in chlorinated chill water (25 ppm), a significant decrease in microbial counts was observed. For pseudomonads, there was an overall reduction in numbers by the end of the chilling process (5 ºC, 40 min, 45 ppm chlorine); however, counts were reduced by less than one log (Allen *et al.*, 2000). A clearer, beneficial effect was obtained with 20 ppm chlorine (Mead *et al.*, 1975) and 25 ppm (Jimenez *et al.*, 2003) for the chiller water itself. Chlorine is largely inactivated in contact with the carcass. Thus, any effect in water chilling systems could be due to an enhanced washing effect (possibly aided by the alkali stabiliser in hypochlorite), or destruction of bacteria in the water, thus preventing re-contamination of the carcasses.

Graw *et al.* (1997a,b) carried out extensive studies on the effects of different air and spray/evaporative-chilling methods on the numbers of spoilage and other bacteria on carcasses. During air-chilling, there was no change in the microbial load of the skin or the visceral cavity. On the other hand, spray/evaporative chilling reduced contamination of the skin by about 0.5 log, but had no impact on organisms in the visceral cavity.

Allen *et al.* (2000) compared several different methods:

- water chilling with chlorinated chill water (45 ppm)
- air chilling
- spray chilling.

For neck skin samples, there was no significant influence of air-chiller type, with or without sprays. In the case of body cavities, however, TVC and counts of coliforms and pseudomonads all fell by approximately one log, when no water sprays were used. With sprays, microbial counts showed less change. Burton and Allen (2002) found no significant overall change (below 0.5 log) in levels of

TVC, coliforms and pseudomonads on the skin during spray chilling, with or without chlorination, and dry chilling. In contrast, the abdominal cavity showed a small increase of up to one log unit, when sprays were used, compared with a reduction by a similar amount in their absence.

Cross-contamination has been considered to be a problem in immersion chilling. However, a study by Mead *et al.* (2000) provided evidence that microbial cross-contamination can also occur during air chilling of poultry, whether or not water sprays are incorporated into the chilling process. Water sprays increased the spread of microorganisms; however, the extent to which they did so may have been exaggerated by deliberately using a heavily-inoculated seeder carcass in the study.

A decrease (or increase) in carcass contamination during chilling is not the only effect of the cooling process. Newell *et al.* (2001) showed that changes in the distribution of *Campylobacter* strains occurred during processing and were most evident after chilling. Indeed, some *Campylobacter* subtypes, but not others, survived chilling and were present on the end-product. Also, Jimenez *et al.* (2003) observed that the proportion of *Enterobacter cloacae* to *E. coli* increased at the chilling step compared with the evisceration and washing stages. Thus, chilling can lead to changes in the microbiological profile of the carcass.

14.4.2 Chilled storage

Traditionally, chickens and turkeys were stored uneviscerated. Grey *et al.* (1986) investigated conditions affecting the keeping-quality of eviscerated and uneviscerated, traditional, farm-fresh turkeys. Initial holding at 4 ºC increased the numbers of psychrotrophic bacteria on the skin by about 10^3 but subsequent changes at −2 ºC were slight for uneviscerated carcasses (Table 14.7). Eviscerated carcasses had higher counts after storage at −2 ºC and, although 'off'-odours were not detected, spoilage appeared to be imminent at the end of the 20-day storage period.

Thomson *et al*. (1975) showed clearly the effect of storage temperature on both TVC (Fig. 14.2) and counts of psychrophiles (Fig. 14.3). TVC from carcasses stored at 5 ºC had reached or were approaching 6 log/cm^2 after 10 days, while those stored at −2 ºC were between 2.5 and 5 log after 37 days. Results for psychrophiles were very similar. The study also showed an effect of

Table 14.7 Changes in microbial counts from turkeys during storage (Grey *et al.*, 1986)

Storage conditions	Eviscerated cfu/cm^2	Uneviscerated cfu/cm^2
Initial birds	4.5×10^2	4.5×10^2
10 days at 4 ºC	1.2×10^5	4.7×10^5
10 d at 4 ºC + 11 d at −2 ºC	4.7×10^5	1.5×10^5
10 d at 4 ºC + 20 d at −2 ºC	1.5×10^7	1.2×10^5

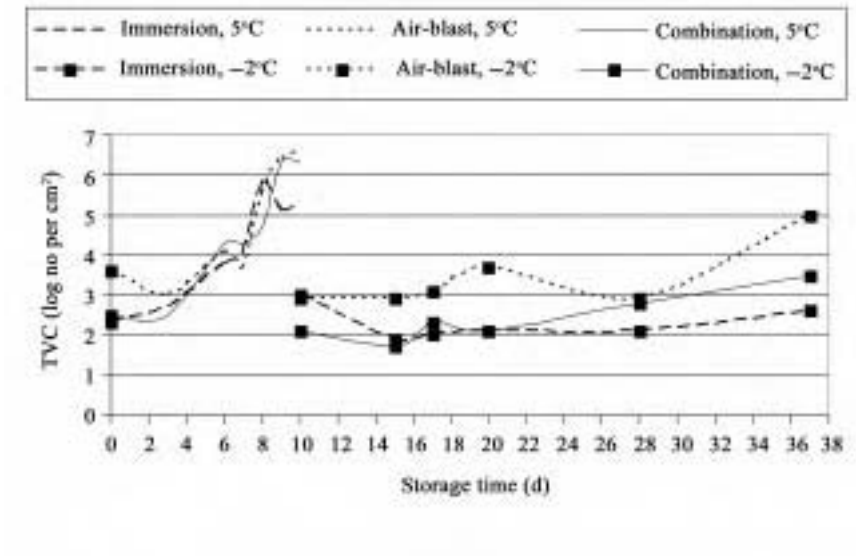

Fig. 14.2 Total plate counts during storage at 5 °C and −2 °C for poultry carcasses chilled by different methods (Thomson *et al.*, 1975).

chilling treatment on storage life. At −2 °C, after 37 days of storage, counts from immersion-chilled carcasses were at least 2 log/cm^2 less than those that had been air-blast chilled. However, Boegh-Soerensen (1979) found that air chilling and counter-flow water chilling resulted in the same storage life.

Different packaging methods and use of modified atmospheres can affect the growth of spoilage organisms on the product during chilled storage, but the

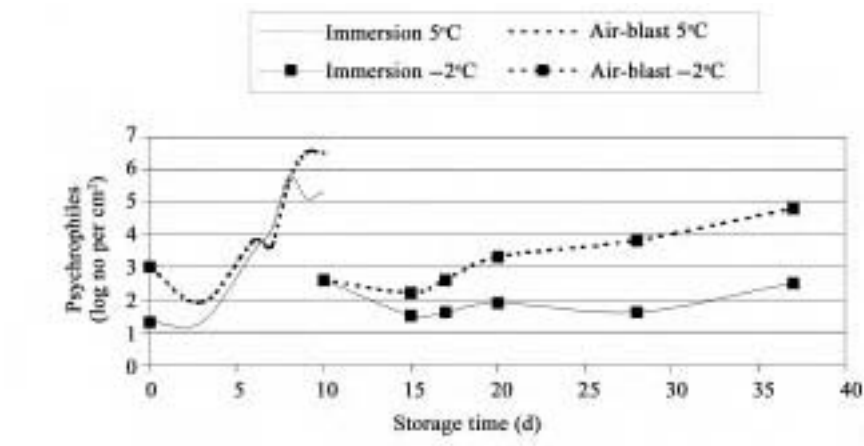

Fig. 14.3 Psychrophilic counts during storage at 5 °C and −2 °C for poultry carcasses chilled by different methods (Thomson *et al.*, 1975).

effect is not the same at all temperatures. Thomson and Risse (1971) found no difference in microbial counts (log TVC/cm^2) from poultry carcasses held at 0.5 ºC for nine days in corrugated fibreboard, expanded polystyrene or wire-bound wooden boxes containing either ice or dry ice. At 4.4 ºC, counts were higher for fibreboard boxes with ice than the other treatments. At 5.2 ºC, counts were lowest in polystyrene boxes with dry ice.

Partman (1978) stored broiler halves in bags of high-density polyethylene for up to six weeks at 1 ºC. The bags were filled with air, pure CO_2 or a 20% CO_2 + 80% N_2 mixture. Initial counts averaged 10^4 per cm^2 for aerobes and 10^3 to 10^4 per cm^2 for anaerobes. After two weeks in air, counts of either kind were above 10^8 and the meat was spoiled. After four weeks in the gas mixture, the counts reached 10^7–10^8, but the meat appeared less spoilt than that held in air for two weeks. After four weeks in pure CO_2, counts of aerobes and anaerobes were only one log higher than their initial values.

Although it is certain that temperature-abuse occurs during chilled storage and distribution of poultry, there is little data on its effect. Russell *et al*. (1992) investigated simulated temperature abuse of ready-to-cook broiler carcasses, which were obtained immediately post-chill from a commercial processing plant. A control group was held at 4 ºC for 10 days. Test groups were held at 4 ºC for two days, then at 10, 15 or 20 ºC for 0, 1, 2, 4, 6, 8, 12 or 24 h, before transferring to 4 ºC for an additional four days. Carcasses were sampled using a whole-carcass rinse technique and TVC obtained at 18 or 42 ºC (Fig. 14.4). Counts from carcasses that had been temperature abused for up to 24 h at 10 ºC were not significantly different ($p < 0.5$) from controls. At an abuse temperature of 15 ºC, counts at 18 ºC were not significantly different after six hours and at 20 ºC after only two hours.

Increasingly, cooked poultry products are chilled immediately after cooking and retailed in a chilled condition. Smith and Alvarez (1988) stored turkey breast rolls that had been vacuum-packed and cooked to an internal temperature of 71 ºC for up to 87 days at 4 ºC. Psychrotrophs were not detected in any core or surface sample during storage, indicating that heating and vacuum-packaging were sufficient to eliminate or prevent the growth of these organisms. Counts of mesophilic anaerobes averaged 188 per g in the core and 100 per g on the surface of the rolls. The anaerobic counts did not change significantly during storage, indicating that the organisms could survive pasteurisation and storage at 4 ºC, but not grow.

There are few published data on microbial contamination of refrigeration components, especially evaporator cooling-coils, in the food industry. Evans *et al*. (2004) measured such contamination (TVC) in fifteen food factories. An initial survey of 891 sites on evaporators, drip-trays and chill-room walls was followed up with a more detailed examination of 336 sites showing high counts, and selecting for *Listeria* spp., coliforms, enterococci, *Staphylococcus aureus* and *Bacillus cereus.* Also surveyed were temperatures (particularly air-on and air-off, maximum and near defrost heaters), relative humidity, airflow, chiller layout and cleaning regimes.

In general, no correlation could be found between any of the physical measurements made and the numbers and types of bacteria detected. Maximum

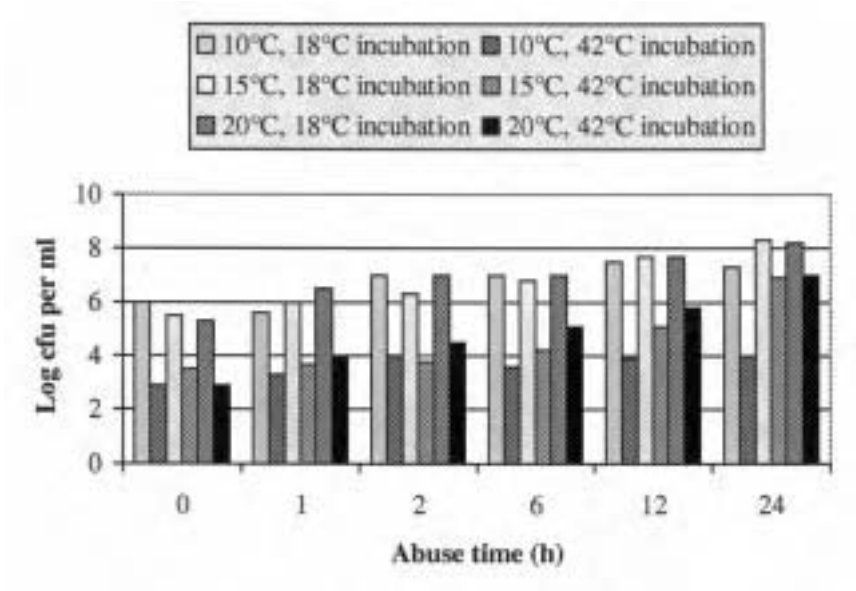

Fig. 14.4 Total plate counts at 18 ºC and 42 ºC for broiler carcasses temperature-abused at different temperatures for different times (Russell *et al.*, 1992).

mean temperatures in the chill rooms varied from −1 to +16.9 ºC and few chilled units were regularly cleaned. Twenty five percent of sites examined had more than 10^5 cfu/cm^2, although very few pathogens or faecal indicator bacteria were detected. *Listeria* spp. were not found and coliforms were found only once, in low numbers. Low numbers of *Staph. aureus* or *B. cereus* were present in nine of the fifteen plants, with *B. cereus* being found on evaporators and associated drip-trays in two plants processing cooked meat. In general, microbial contamination was lower in rooms where wrapped rather than unwrapped products were stored. The type of product also affected the degree of contamination, with raw red meat and poultry or dry ingredients giving the highest counts, and raw vegetables and cooked products the lowest.

The work demonstrated that bacteria were present on evaporator cooling coils in all factory cold rooms visited. Although evaporator cleaning procedures were carried out in some factories as part of routine maintenance, these were shown to be ineffective in keeping counts low. To maintain evaporator hygiene, it was suggested that more regular cleaning, possibly by means of automated cleansing systems, should be considered.

14.4.3 Freezing

Effect of freezing on microorganisms

'Frozen foods have an excellent overall record of safety and illnesses associated with frozen foods are rare. However, in addition to preserving the quality of

foods, freezing also preserves the viability of some pathogenic microorganisms' (Archer, 2004). The author provided some examples of outbreaks and illnesses associated with frozen foods.

In general, freezing results in a small reduction in the numbers of viable microorganisms contained on and within poultry and poultry products. Any organisms present are affected by the low temperature, the formation of ice and the reduction in available water. During the initial stages of the freezing process, as the temperature is reduced, the rate of microbial growth is also reduced and growth ceases below the minimum temperature. In a very long freezing process, however, microbial growth could occur. Lentz and van den Berg (1964) clearly demonstrated the problems of boxing poultry in corrugated cardboard cartons before freezing (Fig. 14.5). Those positioned at the centre of stacks of cartons took three days to freeze and 9 kg turkeys over 10 days. A 5 cm air-gap between the rows reduced the freezing times to 1.5 and 5.5 days respectively.

Some of the psychrophilic organisms may grow at temperatures below 0 ºC and microbial growth can occur in frozen foods stored at −7 ºC. However, it is generally accepted that microbes are unable to grow below −10 to −12 ºC (Mead, 2004).

As the temperature of poultry is reduced below approximately −1.6 ºC, ice crystals will start to form. It is well known that a major factor to consider when assessing how many cells will survive the freezing process is the rate of freezing. Rapid freezing is associated with both intra- and extra-cellular freezing. Slow freezing creates an intra- and extra-cellular osmotic gradient, which results in some cellular disruption (Mazur, 1984). During slow freezing, the product is likely to remain in the region of maximum ice production, −2 to −7 ºC, for an extended period. In a practical situation, there will be a range of

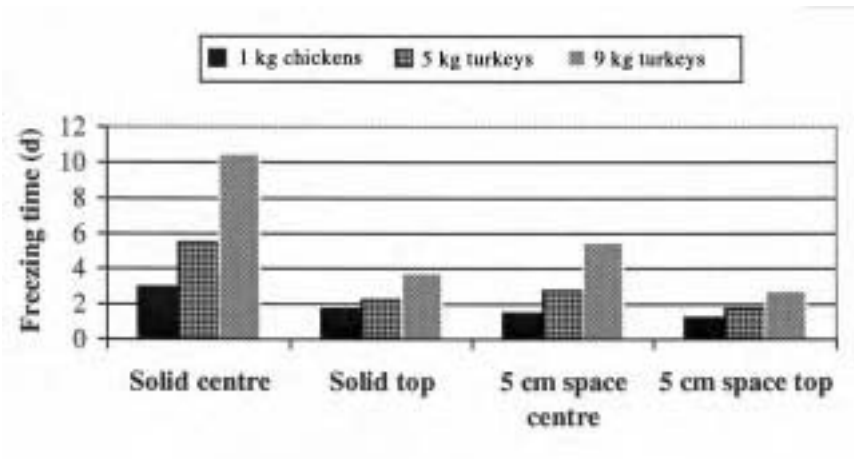

Fig. 14.5 Freezing time of chickens (six per carton in 5 × 3 stack) and turkeys (two per carton in 3 × 3 × 3 stack) in corrugated cardboard cartons (Lentz and van den Berg, 1964).

freezing rates within a poultry carcass or portion and Pickett and Miller (1967) found no difference in microbial survival between hen turkeys frozen rapidly in liquid nitrogen or chilled and frozen in the normal way. However, Foster and Mead (1976) showed that salmonellas added to mined chicken muscle are more sensitive to storage temperatures near the freezing point, such as −2 or −5 ºC, than at much colder temperatures, such as −20 ºC.

Another important factor in freezing is the available water. During freezing, the solutes present become concentrated in the unfrozen portion of the product to a level at which microbial growth is inhibited.

The effects of freezing and thawing on microbial cells can involve physical and oxidative damage (Mazur, 1984; Zhao *et al.,* 2003). Freezing and thawing of living cells results in injury, which has been attributed in part to ice nucleation and dehydration. In addition, oxidative damage has been suggested as a mechanism contributing to freeze-thaw injury resulting from an oxidative burst that occurs within cells during thawing. The results of a study by Parks *et al.* (1998) show that oxidative stress causes major injury to cells during aerobic freezing and thawing, and that this is mainly initiated in the cytoplasm by an oxidative burst of superoxide radicals formed from oxygen and electrons that leak from the mitochondrial electron-transport chain. Duffy *et al.* (2000) reported that freezing results in physical damage and causes a direct increase in the viscosity of the cell as a result of ice-crystal formation. Freezing may also cause some denaturation of cellular proteins (Jay, 1996). Precisely how this is achieved is unclear, but it is known that some –SH groups disappear upon freezing. It is also known that lipoproteins may break apart on freezing. It is reported that the level of cell injury as a result of freezing is very high; however, cells are able to recover and regenerate upon thawing (Budu-Amoako *et al.*, 1992).

The bacterial strain and phase of growth may influence the effects observed. For example, Chan *et al.* (2001) showed that the relative tolerance of *Campylobacter jejuni* to freezing at −20 ºC and freeze-thawing was strain-specific.

14.4.4 Frozen storage

In general, microbial numbers will gradually decrease, if poultry meat is held below −12 ºC. Wills *et al*. (1948) looked at the effects of evisceration practice, packaging and storage temperature on the frozen storage-life of poultry carcasses. The packaging systems used, wrapping in ordinary waxed paper or sealing in tin cans, were not relevant to modern practice. At a storage temperature of −7 ºC, strong odours were detected after three months and mould and slime were observed after six months. At −12 ºC, no sign of microbial activity was detected after nine months. There was no apparent advantage in using storage temperatures below −18 ºC.

Ristic (1982) found a small reduction in TVC over time, when chicken breast muscle was held at −15 or −21 ºC. Log counts per g fell from an average of 6.0 after two months to 5.2 after 16 months.

In less developed countries, poor processing standards and storage conditions can result in high microbial counts from frozen broilers. In Kuwait, Abu-Ruwaida *et al.* (1996a) found relatively high initial counts of *E. coli* and coliforms (average 3.2 and 3.4 log cfu per ml of rinse, respectively), indicating poor sanitation during slaughtering and processing. These counts decreased with increasing time of frozen storage, but the rate of decrease was greater at lower temperatures (−18 ºC) than at higher temperatures (−12 and −5 ºC). *Salmonella* was present in 60–80% of frozen carcasses examined, and this level did not change substantially during prolonged frozen storage. *Campylobacter* and *Staph. aureus* were detected in all carcasses at relatively high levels (mean 2.9 and 4.4 log cfu per ml of rinse, respectively). These levels decreased (by about 1 and 1.5 log, respectively) with extended frozen storage, especially in carcasses stored at lower temperatures (−18 ºC). Freezing and/or prolonged frozen storage generally reduced, but did not render the meat completely free from microbial contaminants.

In imported frozen broilers, Abu-Ruwaida *et al.* (1996b) found low initial mean counts of psychrotrophic organisms (2.6–3.4 cfu per ml of rinse). At −12 ºC, counts did not change during nine months of frozen storage; however, the number of viable bacteria decreased slightly in carcasses stored at −18 ºC. Initial counts of coliforms were relatively low and decreased further during storage. Pathogens, such as coagulase and DNase-positive *Staph. aureus*, *Salmonella* and *C. jejuni* were also detected in imported poultry meat, but their levels were far below those recorded in local (Kuwait), frozen poultry meat.

C. jejuni is a leading cause of acute bacterial gastroenteritis throughout the world, with poultry as a major vehicle of human infection. Zhao *et al*. (2003) inoculated a mixture of three strains of *C. jejuni,* originally isolated from poultry, onto chicken wings at about 10^7 cfu per g. After 72 hours at −20 and −30 ºC, the population of *C. jejuni* on the wings was reduced by 1.3 and 1.8 log cfu per g, respectively. When the meat was stored for 52 weeks at −20 ºC, *C. jejuni* was reduced by approximately 4 log cfu per g; however; at −86 ºC only a 0.5 log cfu per g reduction was observed. Yogasundram and Shane (1986) found that an initial level of *C. jejuni* contamination on chicken drumsticks of 9.9×10^2 cfu per cm^2 declined to 4.5×10^1 cfu per cm^2 after 7 days at −20 ºC. Thereafter, *C. jejuni* persisted at levels ranging from 1.8×10^1 to 0.2×10^1 cfu per cm^2 through 26 weeks of frozen storage.

In Iceland, carcasses from farms that tested positive for *Campylobacter* spp. were subsequently frozen prior to distribution. Stern *et al*. (2003) considered this one of the main factors contributing to a reduction in poultry-borne campylobacteriosis in that country. Chan *et al*. (2001) found that the relative tolerance of the organisms to freezing at −20 ºC and freeze-thawing was strain-specific, but independent of strain source (poultry versus clinical) and degree of tolerance to 4 ºC exposure.

The effect of frozen storage on a number of other pathogens has been investigated. Gianfranceschi and Aureli (1996) found a slight decrease (range 0.1–1.6 log) in the viability of *L. monocytogenes* (Scott A and FIL/IDF strains)

inoculated onto chicken breast, after 57 minutes at −50 ºC. Following approximately 9 months in frozen storage at −18 ºC, a further reduction of up to 1.0 log was observed. During storage of broilers at −18 ºC for up to 6 months, the numbers of cells of *S. typhimurium, S. meleagridis* and *S. gallinarum-pullorum* decreased steadily (Georgiev *et al*., 1978); however, some survived. The reduction in count was greatest in the first 15 days. Among the organisms tested, *S. typhimurium* was the most resistant to the effect of low temperature and *S. meleagridis* the least resistant.

14.4.5 Thawing

Thawing has received much less attention in the literature than either chilling or freezing. In commercial practice, there are relatively few controlled thawing systems.

Frozen poultry meat, as supplied to the industry, can be in the form of whole carcasses, bone-in portions or boned-out packs in cartons. Thawing is usually regarded as complete when the centre of the meat has reached 0 ºC, the minimum temperature at which the meat can be deboned or cut by hand. Lower temperatures (e.g. −5 to −2 ºC) are acceptable for meat that is destined for mechanical chopping, but such meat is 'tempered' rather than thawed. The two should not be confused, because tempering only constitutes the initial phase of a complete thawing process.

Thawing is often considered to be simply the reversal of the freezing process. However, inherent in thawing is a major problem that does not occur in the freezing operation. The majority of bacteria that cause spoilage or food poisoning are found on the surface of meat. During the freezing operation, surface temperatures are reduced rapidly and bacterial multiplication is severely limited, with no growth at all below −10 ºC. In the thawing operation, these same surface areas are the first to rise in temperature and microbial multiplication can then recommence. On large objects subjected to long, uncontrolled thawing cycles, surface spoilage can occur before the centre regions have fully thawed.

Most thawing systems supply heat to the surface and then rely on conduction to transfer that heat into the centre of the meat. A few use electromagnetic radiation to generate heat within the meat. In selecting a thawing system for industrial use, a balance must be struck between thawing time, product appearance, final bacteriological condition of the product, processing problems, such as effluent disposal, and the capital and operating costs of the respective systems.

There is little published data on the microbial changes occurring during the thawing of poultry meat. However, the data on thawing of red meat (James and James, 2002) indicates that higher thawing temperatures result in greater microbial growth. Buttiaux (1972) reported that water-thawing was more successful for beef than for pork, if the meat was to be stored. Consequently, care must be exercised in extrapolating from one meat species, to another. In a well-controlled tempering operation, there should be no opportunity for growth of pathogenic or food-spoilage organisms.

C. jejuni 81116 was inoculated onto chicken skin by Lee *et al*. (1998) and subjected to repeated freeze-thaw cycles. The organism was found to withstand repeated freezing and thawing, similar to those procedures that may occur in the home. Under all freezing conditions, the *C. jejuni* strain retained a high level of viability and quickly replicated to levels that exceeded microbial levels on raw food products that are permitted in Australia, after thawing.

It is often asserted that thawed food is more perishable than fresh or chilled items, but experiments (Kitchell and Ingram, 1956, 1959) have failed to demonstrate any difference of practical significance between the growth of meat-spoilage organisms on fresh and thawed slices of meat. Greer and Murray (1991) found that the lag phase of bacterial growth was shorter in frozen and thawed pork than in fresh meat, but the generation time was unaffected.

In the home situation, it is sometimes suggested that poultry should only be thawed in a refrigerator. For example, Maciorowski *et al*. (1999) stated that 'A majority of Hispanics (57%), miners (79%) and uneducated (55%) respondents thaw poultry outside of the refrigerator, either on a counter, in a microwave or in a sink of water.' In another survey, Mitakakis *et al*. (2004) reported that chicken was thawed using unsafe means by 76.3% of respondents and Sammarco *et al.* (1997) stated that 73% of respondents thawed large pieces of frozen food at room temperature. However, Lacroix *et al.* (2003) advised caution about making recommendations to consumers that were not based on firm scientific evidence. When thawing turkeys in the home, it was suggested that contamination of the work area is the major concern. 'While several methods, including thawing on the counter at ambient temperature, can be employed for thawing turkey, cooking to an adequate internal temperature, validated with a meat thermometer, is the more critical step.'

14.4.6 Transport, retail display and consumer handling

Developments in frozen transport in the nineteenth century established the international food market, when the first cargo of frozen meat was sent from Buenos Aires to France in 1877. There are few problems in the long-distance transportation of poultry meat, as long as the meat is at the correct temperature before loading, since the refrigeration systems used in most transport containers are not designed to extract heat from the load, but merely to maintain its temperature. In the large containers used for transportation, meat temperature can be kept within $\pm 0.5\,^{\circ}C$ of the set point. With this degree of temperature control, long-distance transportation times of eight to fourteen weeks (for vacuum-packed meats stored at $-1.5\,^{\circ}C$) are possible, while providing a sufficient chilled storage-life for retail display.

In contrast, there are substantial difficulties in maintaining the temperature of refrigerated meat when transported in small, refrigerated vehicles that conduct multi-drop deliveries to retail stores and caterers. During any one delivery, the chilled product can be subjected to as many as 50 door-openings, where there is heat ingress directly from outside and from personnel entering to select and

remove product. The design of the refrigeration system has to allow for extensive differences in load distribution, dependent on different delivery rounds, days of the week and the removal of product during a delivery run.

On progressing through the cold chain, it becomes increasingly difficult to control the temperature of poultry meat and meat products. In particular, it is more difficult to maintain the temperature of a retail display cabinet or domestic refrigerator than a large storage room. Being smaller, retail packs respond more rapidly to temperature fluctuations than bulk packs. The desired, chilled display-life for wrapped meat and meat products ranges from a few days to many weeks and is limited primarily by microbiological considerations. Retailers of unwrapped meat and delicatessen products, e.g. sliced meats and pâté, normally require a display life of one working day. Frozen products, on the other hand, can remain on display for many weeks.

Chilled chicken products can be subjected to temperature-abuse during commercial transportation, retail storage, transfer of the product to the home and storage in domestic refrigerators. Evans *et al*. (1991) measured temperatures as high as 28 ºC in cooked chicken and over a set period predicted increases of up to 1.8 generations in bacterial numbers (Table 14.8). Surveys since 1990 have revealed overall mean temperatures in domestic refrigerators ranging from 4.5 to 6.6 ºC (James, 2004). After five days of storage in a domestic refrigerator, Conner *et al*. (1953) found that microbial numbers on fresh turkey steaks had risen from 5×10^3 to over 1.1×10^6 cfu per g. Numbers on frozen steaks that had been thawed and stored in the same refrigerators reached 4.2×10^5 cfu per g after five days.

Home produced, chicken-based foods may be especially susceptible to temperature abuse. Erickson *et al*. (1993) prepared home-made chicken salads with three different commercial mayonnaise products:

1. real mayonnaise
2. reduced-calorie mayonnaise dressing
3. reduced-calorie/reduced-fat mayonnaise dressing.

The salads were inoculated with 10^3 per g levels of *Salmonella* or *L. monocytogenes* and held at 4 ºC and 12.8 ºC for ten and two days, respectively.

Table 14.8 Maximum temperature measured and predicted increase in microbial numbers in chilled foods during one hour in a car followed by five hours in a domestic refrigerator (source: Evans *et al*., 1991)

Product	Conditions	Maximum temperature (ºC)	*Pseudomonas* (generations)	*Clostridium* (generations)
Chicken, raw	Ambient, car	24	1.6	0.2
	Cool box, car	4	0.0	0.0
Chicken, cooked	Ambient, car	28	1.8	0.7
	Cool box, car	12	0.0	0.0

Growth of *Salmonella* occurred only at 12.8C, while *L. monocytogenes* grew at both temperatures. The synergistic combination of mayonnaise and refrigeration inhibited outgrowth of *L. monocytogenes* for more than seven days. No microbiological safety or spoilage differences were observed between the salads prepared with real mayonnaise or reduced-calorie mayonnaise dressings. It was concluded that, under proper refrigeration and good hygiene practices, home-style salads made with commercial, real mayonnaise or mayonnaise dressings represent negligible health risks to consumers from microbial growth.

14.5 Improving control in the cold chain

In general, temperature control for frozen poultry is sufficient to prevent any microbial growth and, in most cases, microbial levels are reduced as poultry progresses through the supply chain. However, work is needed to improve control of thawing processes, both in industry and by consumers.

The production of an increasing range of 'fresh', chilled poultry portions is more problematical. Greater handling increases the risk of contamination and cross-contamination. Reducing meat temperature and keeping the meat close to its final factory temperature will reduce any consequent risk. Future developments should concentrate on improved primary chilling, retail display and domestic refrigeration of poultry.

14.6 Future trends

The frozen sector of the poultry industry is likely to be increasingly dominated by imports from South America and Asia, where production costs are significantly lower than in the major consumer markets. Frozen boned-out chicken is likely to form the main raw material for the production of all pre-cooked chicken products. If produced hygienically, frozen immediately after production, maintained below $-12\,^{o}C$ and either tempered before use or cooked directly from frozen, use of this material should minimise any risk of food poisoning.

In the developed world, the 'perceived' quality of a 'fresh', chilled product over a frozen one is likely to continue. The 'perceived' view in the industry that air chilling is preferable to immersion systems is also likely to continue. If surface-freezing is to be avoided in a chilled product, then an immersion system, especially one using an ice-water mixture will always be faster and less problematical than an air-based process. To meet the economic need for ever higher throughputs, multi-stage chilling systems, using high-velocity, low-temperature air in the initial stages, are likely to become common practice. Poultry meat will be chilled to and maintained at temperatures close to the initial temperature throughout distribution and subsequent holding. This will result in extended, 'safe' shelf-lives. However, substantial improvements in temperature control are needed during retailing and consumer handling to maximise these benefits.

14.7 Sources of further information and advice

International Institute of Refrigeration, 177 Boulevard Malesherbes, F-75017 Paris, France. Tel.: +33 1 4227 3235, http://www.iifiir.org.

Food Refrigeration and Process Engineering Research Centre (FRPERC), University of Bristol, Churchill Building, Langford, North Somerset, BS40 5DU, UK. www.frperc.bris.ac.uk.

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15

Sanitation in poultry processing

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15.1 Introduction

This chapter explores the concerns of processors, consumers and others interested in food safety regarding sanitation and hygiene in poultry-processing facilities. Sanitation and process-control programs help to ensure the safety and quality of the food being produced; they can also result in a longer shelf-life and encourage repeat purchases (Ollinger and Mueller, 2003). The Hazard Analysis Critical Control Point (HACCP) system is designed to prevent the occurrence of hygiene problems by ensuring that controls are applied at any appropriate point in the production process, and control of plant sanitation is an integral part of the system. Establishments that produce meat and poultry products are required to develop plans for controlling any food safety hazards that can affect their products. Strategies for intervention in the processing plant are aimed at the prevention of contamination and inactivation of foodborne pathogens. Current technologies for common sanitation practices include physical, chemical and biological strategies for reducing bacterial contamination in general and the presence of pathogens in particular. Good cleaning is a prerequisite of effective sanitation in all situations. Substantial bacterial contamination of the poultry-processing environment, e.g. carcasses and plant surfaces, involves the attachment over time of many different species of microbes to other microbes, debris and inert surfaces. To implement control measures effectively, management must provide the necessary resources – training, education and money. High-quality sanitation and process-control programs raise the cost, but losing a reputation for producing safe products can be even more costly.

15.2 Sanitation programs

15.2.1 The HACCP system

Food safety is a particularly difficult product attribute to convey to consumers, because it cannot be observed directly. Consumers learn about this attribute by either eating the food themselves or by observing the consequences of it being eaten by others. Public fears over the wholesomeness of meat and poultry products accelerated during the 1980s. In response, regulations requiring safe handling of meat and poultry have increased. Throughout the European Union, HACCP is a mandatory system used by all food companies and the majority of US food companies are now implementing HACCP, whether mandated or not (Sperber, 2004).

The Food Safety and Inspection Service (FSIS) is the regulatory agency within the Department of Agriculture that ensures safe food products in the USA. Its directives require that establishments meet a set of standard operating procedures (SOPs) for sanitation and certain performance standards (FSIS, 2003). Each establishment must develop, implement and maintain written procedures for the action it takes daily, before and during operations, to prevent the product from being directly contaminated and adulterated. Typically, the procedures cover the scheduled, daily pre-operational and operational cleaning and sanitation of equipment and surfaces that may come into direct contact with the product. The performance standards cover pest control, ventilation, lighting and plumbing systems.

The HACCP system is designed to prevent the occurrence of problems, firstly by identifying and evaluating any potential hazards. These include biological, chemical or physical contamination of food products. The 1998 rule requires that establishments must develop and implement written SOPs for sanitation, develop and implement a HACCP system, meet *Salmonella* performance standards established by FSIS testing, and conduct routine testing of carcasses during processing for generic *Escherichia coli*, as an indicator of fecal contamination.

15.2.2 HACCP plans

Establishments that produce meat and poultry products are required to develop a plan for controlling food safety hazards that can affect their products. According to FSIS theory, if the plan they design is effective in eliminating health and safety hazards and, if the establishment executes the plan properly, then the resulting product should be safe for consumers (FSIS, 1998). For a poultry processing plant, a model plan can include process flow diagrams, product descriptions, hazard analysis forms, records of re-processing inspections, details of the chilling process, thermometer calibration, room temperature control, antimicrobial intervention monitoring, any corrective action and pre-shipment reviews.

15.3 Sanitation technologies

15.3.1 Bacterial contamination and biofilm formation

Understanding the conditions conducive to bacterial attachment and biofilm formation will provide important information for successful development of sanitation plans for poultry-processing plants. The phenomenon of bacterial attachment to surfaces, such as metal, rubber and plastics, presents a formidable obstacle to surface cleaning and sanitizing treatments (Taylor, 1970; Wirtanen and Mattila-Sandholm, 1992; Arnold, 1998). When bacterial cells attach initially to a surface, they produce extracellular fibrils that form a complex matrix favoring the attachment and subsequent growth of more bacteria, while attracting other microbes and debris as well (Arnold and Shimkets, 1988a; Steinberg and Poole, 1981). The ultimate composite is a biofilm that is resistant to cleaners and sanitizers, and is extremely difficult to remove (Zottola, 1994).

Much research on bacterial contamination of stainless steel surfaces has been conducted in relation to cleanability and disinfection (Stone and Zottola, 1985; Holah and Thorpe, 1990; Krysinski *et al.*, 1992). The resistance or susceptibility of the surface to bacterial contamination has been given little attention, however, in the manufacturing of food processing equipment. Now, HACCP plans for poultry production and processing are giving a new impetus to this consideration (Anon., 1996). If microorganisms on food-contact surfaces are not completely removed, they may lead to biofilm formation and also increase the potential for cross-contamination (Hinton *et al.*, 2003). Control of biofilm formation depends on hygienic plant layout and design of equipment, choice of suitable materials, correct selection and use of detergents and disinfectants, coupled with appropriate physical cleaning methods (Kumar and Anand, 1998).

Substantial bacterial contamination of the poultry-processing environment (carcasses and plant surfaces) occurs over time and involves many different microbial species. Organic and inorganic material in the liquid phase can sediment onto solid surfaces. Subsequently, biologically active microorganisms will be attracted to these conditioned surfaces and adhere. The complex community that forms soon develops into a a biofilm (Fig. 15.1). Bacteria are also attracted to the interfaces between surfaces (Arnold and Shimkets, 1988a; Hermanowicz *et al.*, 1995), and biofilms occur most readily on solid surfaces that are wet (Costerton *et al.*, 1987; Zottola, 1994). In a recent study of 41 meat plants, *Listeria monocytogenes*, one of the meat industry's most tenacious pathogens, was found on or in 39% of the floors tested, 29% of floor drains, 34% of cleaning equipment, 24% of wash areas and 20% of food-contact surfaces (Bjerklie, 2003). Every one of these sites is a haven for biofilm formation.

In general, biofilms are known to be more resistant to disinfectants than planktonic suspensions of laboratory cultures of single organisms. Under biofilm conditions, bacteria become more resistant to both physical and chemical treatments used in plant sanitation practices (Arnold, 1998). Pathogens within biofilms may be able to survive better against plant cleaning procedures than

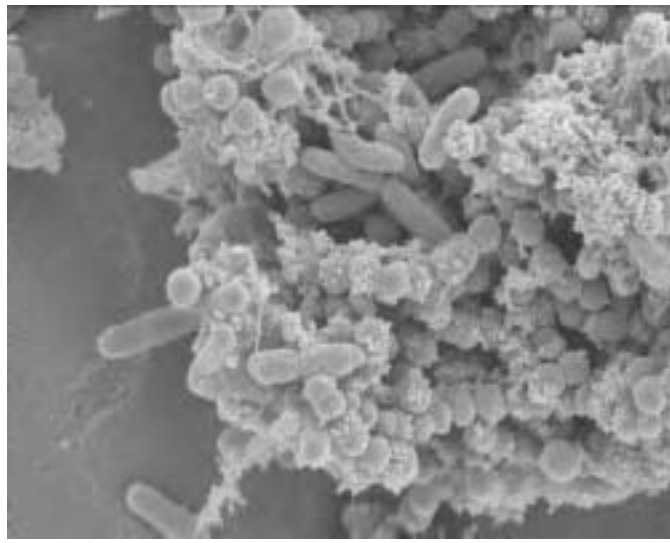

Fig. 15.1 Biofilm on stainless steel. Scanning electron micrograph of stainless steel exposed to a mixed population of bacteria taken from rinse-sample of whole broiler carcass, after two hours exposure. Magnification = 10 000×.

was previously thought, although, the resistance to disinfectants of newly recognized pathogens, such as *Arcobacter*, has not been documented.

Wirtanen and Mattila-Sandholm (1992) found that tolerance to chlorine and heat treatment among *Listeria* spp. and other microorganisms in biofilms is increased after attachment. Oh and Marshall (1995) obtained similar results with monolaurin and heat treatment. Dhir and Dodd (1995) found that attached cells of *Salmonella* Enteritidis were more than twice as resistant to heat treatment as planktonic cells. Somers *et al*. (1994) showed that attached cells of *Campylobacter jejuni*, *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium were more resistant than planktonic cells to trisodium phosphate treatment. Iodophor, hypochlorite, anionic acid, peroxyacetic acid, fatty acid and quaternary ammonium sanitizers were relatively ineffective against attached bacteria during milk processing (Mosteller and Bishop, 1993). Biofilm and planktonic listerias also reacted differently to the removal of microbial nutrients from surfaces. Nutrient deprivation reduced the susceptibility of planktonic listerias to benzalkonium chloride, but had no effect on the more-resistant biofilm cells (Ren and Frank, 1993).

15.3.2 Processing surfaces that come into contact with the product

Surface characteristics – structure, chemical composition and electronic properties – control the chemical reactivity of surfaces (Hochella *et al.*, 1989). In turn, these characteristics influence the binding of substrates, including bacterial extracellular polymer, to surfaces. Surfaces are heterogeneous in

relation to properties that influence sorption and binding among other types of surface reactions, e.g. nucleation, crystal growth, precipitation and dissolution. Atomic steps, edges, dislocations and point defects determine the heterogeneity of surfaces, including metal surfaces. Edge sites and steps of minerals (Zhang and Bailey, 1998) are more reactive than basal surfaces for adsorptive reactions.

Historically, the use of rubber fingers on mechanical pickers to remove feathers from scalded broiler-chicken carcasses has been considered a major contributor to cross-contamination (Lillard, 1986; Dodd *et al.*, 1988). Contaminating bacteria come ultimately from the birds, with organisms that are attached to, or entrapped in, the skin (McMeekin *et al.*, 1984; Kim and Doores, 1993). During the defeathering process, the intact skin can be damaged, allowing bacteria to become lodged underneath and proliferate (Thomas and McMeekin, 1980; Lillard *et al.*, 1987). However, the results of some studies have shown that new picker-finger rubber inhibits microbial contamination (Arnold and Silvers, 2000). When a mixed population of bacteria was collected from broiler carcasses on a poultry processing line, scanning electron microscopy (SEM) confirmed that new picker-finger rubber could inhibit bacterial attachment and biofilm formation (Fig. 15.2). Furthermore, bacterial growth rate in the presence of the rubber was less than that of controls or other surfaces. Surface composition can control the reactivity of the surface (Hochella *et al.*, 1989), influencing the binding of substrates, including bacterial extracellular polymers. Some of the elements in particular materials are susceptible to bacterial attachment. Elements such as iron, manganese and calcium are known to enhance attachment. However, sulfur and zinc, which are primary elements in rubber, are common antimicrobials. In practice, bacterial adhesion cannot be explained entirely on the basis of the elements present, because polyethylene contains sulfur and zinc as primary elements, but nevertheless appears to be as susceptible to bacterial attachment as other materials. Some other inhibitory factor exuding from the rubber may be contributing to the inhibition.

Bacterial attachment to picker-finger rubber appears to be inhibited initially. However, after the fingers have become worn, cracked or covered with dirt and feces, they can provide favorable growth conditions for bacteria in the warmth and moisture of the defeathering machinery (Kim and Doores, 1993). Bacteria grow readily on organic detritus, and could easily become buried in the crevices and ground into the skin or flesh of a carcass.

Methods have been developed to measure attached bacteria and to identify factors that make surface finishes susceptible or resistant to bacterial attachment and biofilm formation (Arnold and Bailey, 2000). Samples of the treated surfaces (sandblasted, sanded or electropolished) were exposed to natural bacterial populations from chicken-carcass rinses, and bacterial growth and biofilm were allowed to develop on the surfaces. The kinetics of bacterial growth during surface exposure was followed by UV-visible spectrophotometry and counts of bacteria and early biofilm formation were measured with the aid of SEM. The surface morphology of the samples was analyzed by atomic force microscopy (AFM), using samples from each of the treatments examined in the SEM studies.

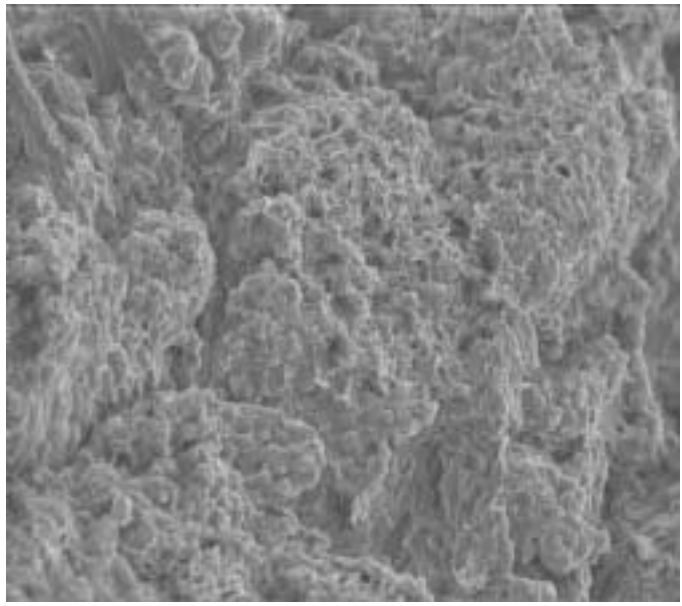

Fig. 15.2 Picker-finger rubber. Scanning electron micrograph of the rubber, when exposed to bacteria under the same conditions as Fig. 15.1. No bacteria are present. Magnification = 3000×.

Relative differences in surface morphology, including fractal dimensions, Z ranges, roughness and other measurements corresponded by treatment with the differences in bacterial counts, as shown by SEM. The surface types varied in affinity for bacteria, and both physical and electrochemical treatments improved the resistance of stainless steel to bacterial attachment. Electropolished stainless steel was the least rough surface and showed significantly fewer bacterial cells or initial biofilm formation than the other treated surfaces.

Corrosion, an important factor in the durability of a metal finish after exposure to water and chemicals, is a real concern for many wet-process industries. The effects of rouging, corrosion and biofouling on the surface of stainless steel, the most common material in processing plants, are costly problems. A corrosive treatment that is indicative of the wet conditions commonly found in food processing was used to test the effect of surface corrosion on bacterial attachment (Arnold and Suzuki, 2003). The kinetics of bacterial growth during surface exposure were determined as described above, and counts of bacteria and early biofilm formation were determined with SEM. Exposure to the corrosive treatment affected bacterial attachment in each case. After exposure, significantly greater numbers of bacteria attached to steel-ball burnished (Fig. 15.3(a)) and glass-beaded finishes (Fig. 15.3(b)); however, the untreated control finish (Fig. 15.3(c)) and electropolished samples (Fig. 15.3(d)) had fewer attached bacteria. Electropolished samples were significantly more resistant, before and after exposure to corrosive treatment, than seven other finishes tested.

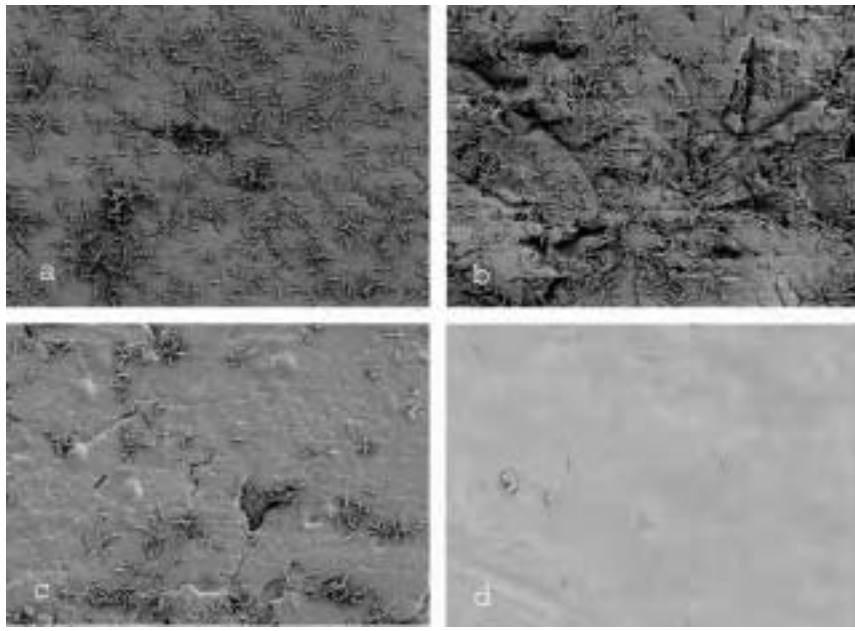

Fig. 15.3 Scanning electron micrograph of stainless steel surfaces after exposure to corrosive conditions and bacteria. Significantly greater numbers of bacteria are attached to steel-ball burnished (a) and glass-beaded finishes (b). The untreated control finish (c) and electropolished samples (d) had fewer bacteria attached. Electropolished samples were significantly more resistant, before and after exposure to corrosive treatment, than the other finishes tested.

Food safety could be improved if bacterial populations on processing equipment could be reduced by greater use of materials that are resistant to corrosion, bacterial contamination and biofilms formation. The above findings could aid equipment manufacturers and processors in selecting more suitable materials and finishes.

15.3.3 Physical methods to prevent or remove contamination

Physical treatments, such as steam, water-pressure and heat treatments, are all used to reduce microbial contamination in food plants (Arnold, 1998). Pretreatment of stainless steel surfaces, as described above, can also reduce bacterial attachment and biofilm initiation. Stainless steel, although susceptible to bacterial attachment, is the most frequently used material for the construction of vessels, piping, valves and various types of equipment used in the food-processing industry.

As discussed above, bacterial contamination and early biofilm formation on stainless steel surfaces can be reduced by both mechanical and electrochemical treatments that affect surface reactions. Visual observation, however, can be misleading in relation to the smoothness and cleanability of a surface. Although

Table 15.1 Roughness data for stainless-steel surface finishes[a]

Surface Finish	Ra range (μm)
Untreated[b]	105–150
Sandblasted[c]	110–475
Glass-beaded	235–255
Burnished[d]	70–90
Electropolished	8–10

[a] These data are derived from 50 samples with replicates. The samples were supplied by four steel manufacturers, and each of the treatments represents samples from at least two equipment manufacturers (eight in total).
[b] 304-2b 11 gauge stainless steel, 1 × 4 cm, most common in poultry processing.
[c] Size of particle and force are critical factors.
[d] Sometimes called steel-ball burnished, steel-shot burnished, or shot peened.

each of the treated surfaces described above was less susceptible to bacterial attachment and biofilm development than untreated stainless steel, electropolished samples showed significantly fewer bacterial cells and initial biofilm formation than the others tested. Bacterial adhesion results from an interplay of forces, including van der Waals, electrostatic and hydrophobic interactions (Arnold and Shimkets, 1988b; Arnold *et al.*, 1993; Ong *et al.*, 1999). Electropolishing removes metal from the surface through an electrochemical process similar to, but the reverse of, electroplating. Removal of metal ions reduces the chemical reactivity of the surface, rendering the surface less susceptible to bacterial attachment.

Several imaging studies that included AFM, SEM and electron-probe microanalysis (EPMA) have shown the relationships between bacterial attachment and biofilm formation, and between surface reactivity and roughness (Arnold and Bailey, 2000; Arnold and Silvers, 2000; Arnold and Suzuki, 2003). The ranges of the roughness average (Ra)/root mean square for treatments of stainless steel in these studies are shown in Table 15.1. Resistance or susceptibility of a surface to bacterial contamination correlated with the roughness factors. Increased bacterial contamination and biofilm formation occurred with increased roughness.

Decreases in the AFM measurements of roughness parameters for the treated surfaces corresponded with the reduction of bacterial contamination and early biofilm formation shown by SEM. AFM is a useful tool to predict the potential for bacteria to attach and form biofilms on surfaces, without the time-consuming sample preparation and tedious counting required for SEM. The design of materials aimed at reducing microbial contamination of surfaces during food processing requires an understanding of the forces of bacterial attachment and biofilm formation.

15.3.4 Chemicals for controlling microbial growth

Possible chemical agents include several groups of substances that destroy or limit microbial growth on food surfaces or inanimate objects. The major groups

are phenolics, halogens, alcohols, heavy metals, surfactants, quaternary ammonium compounds (quats), organic acids, enzymes, certain gases and oxidizing agents. Most cleaning products on the market today contain some combination of these chemicals as active ingredients, depending on the organic matter present on the surface to be treated (Arnold, 2003). The following describes a research approach for the testing of two specific products, and gives brief details of the more commonly used compounds. However, many commercial variations have been tested on different microbes (Bellinger, 1987; Best and Coates, 1990; Wirtanen *et al.*, 1998; Stewart *et al.*, 2001), and therefore the list is not intended to be complete.

Products with formulations that include multiple agents are generally used in plant cleaning and disinfection. Disinfectants are used on hard inanimate surfaces and aim to destroy or inactivate fungi and bacteria, but not necessarily their spores. One such product, with an iodine base, was screened for its effectiveness in controlling bacterial growth and attachment (Arnold and Yates, 2002). The product was tested against various bacterial species, including several important pathogens. In laboratory assays, the product was effective against strains of *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *L. monocytogenes*, *Staphylococcus aureus*, *S.* Enteritidis, and *E. coli*. A subsequent goal has been to target the early stages of poultry processing, especially stainless steel equipment and defeathering machinery, because of the stability of the product at high temperatures. Experiments with rubber picker fingers from three processing facilities showed that the product was effective in controlling bacterial contamination on this type of surface.

The use of a silver-based product was more effective in the later stages of the processing operation. The activity and stability of the product were measured against bacterial growth in both planktonic and sessile conditions. The tests included measurement of residual disinfectant activity. The formulation was effective against *L. monocytogenes*, *Staph. aureus*, *S.* Enteritidis, and *E. coli*. The corrosion rate on stainless steel was also determined for this product. Corrosion rate affects the useful life of equipment and ease of contamination and cleaning. Results from the experiments indicate that the product would be useful in practice. As a general ‘rule of thumb’, the processing industry requires disinfectant products that provide a four-log kill or better.

Sanitizers are used to reduce, but not necessarily eliminate, microorganisms from inanimate surfaces, reaching levels that are considered safe by public health authorities. Sanitizers also include food-contact products, which are used for items such as dishes and cooking utensils, as well as equipment and utensils found in dairies and food processing plants. These products are designed to enhance the duration of disinfectant activity on pre-cleaned surfaces.

Oxidizing agents are most commonly used in product-contact areas and in product rinses and sprays. The use of chlorine has come under scrutiny, because of its potential to react with natural organic matter and form chlorinated by-products that may present a health hazard. Alternative options that show promise are chloramines, chlorine dioxide and ozone. Chloramines have the significant

advantage of virtually avoiding the formation of chlorinated by-products and, unlike chlorine, do not react with phenols to create taste- and odor-producing compounds. However, the presence of nitrifying bacteria and production of nitrite and nitrate in chloraminated water distribution systems, as well as the formation of organic chloramines, have raised concern.

Chlorine is used in the form of gaseous chlorine or hypochlorite. In either form, it is a potent oxidizing agent that reacts with a wide variety of compounds, in particular those that act as reducing agents (hydrogen sulfide, manganese, iron, sulfite, bromide, iodide and nitrite) (Amy *et al.*, 2000). Chlorine can be added to product rinses and chiller vats and is supplied as chorine gas or calcium or sodium hypochlorite. The upper limits of use are restricted by regulatory, export and product-quality issues. The levels required depend on the quality of the incoming water and the amount of suspended organic solids (fat, blood and protein) from processing (White, 1999).

Trisodium phosphate (TSP) was used first in 1997 to reduce microbial levels on chilled poultry carcasses, as an aid in re-processing. Now, many plants use it in combination with chlorine in inside/outside bird washers and as a final rinse. It is applied at a concentration of 8–12% at a pH of 11. Treated carcasses tend to have a more yellowish hue than normal. There is a view that TSP can be replaced by sodium metasilicate, which uses the same equipment and process controls, but does not contain phosphates that are difficult to dispose of effectively.

Chlorine dioxide is one of the few compounds that exist almost entirely as monomeric free radicals. The concentrated vapor is volatile and potentially explosive, so the gas must be manufactured at the point of use. Neutral or acidic, dilute aqueous solutions are stable, if kept cold, sealed and protected from sunlight. Chlorine dioxide is a powerful oxidizing agent, reacting with iodide, sulfide, iron and manganese. Unlike chlorine and ozone, treatment with chlorine dioxide will not transform the bromide ion into hypobromite and will not give rise to bromoform or bromate (Amy *et al.*, 2000). Application is usually confined to a spray cabinet.

Ozone is a strong oxidizing agent with more complex reactions. Ozone may react directly with dissolved solutes or decompose, forming highly reactive secondary oxidants, such as hydroxyl radicals. These radicals and their reaction products can actually accelerate the decomposition of ozone. Many applications of ozone have been developed for the oxidation of organic compounds.

Chloramines, especially monochloramine, are poor oxidants and primary disinfectants. Because of its persistence, monochloramine is useful as a secondary disinfectant, providing a stable residual for water-distribution systems (Amy *et al.*, 2000). The use of ozone or chlorine dioxide combined with chloramines, as secondary disinfectants, has been attractive for minimizing disinfectant by-product formation (Singer, 1994).

Substrate-utilization data may be useful in characterizing and controlling biofilm communities implicated in pathogenicity or affecting the quality of chicken meat. Reduction or removal of rapidly-metabolizable substrates in the poultry processing environment may reduce biofilm formation and persistence.

Candidate substrates may include some of the 17 individual substances with a frequency of utilization that differed by more than 30% between biofilm communities associated with meat samples stored at either 4 ºC or 13 ºC (Boothe *et al.*, 1999; Boothe and Arnold, 2001).

15.3.5 Reducing contamination in air and on surfaces by negative air ionization

Safety concerns about the use of chemical methods for removing and killing bacteria during plant sanitation could be reduced by the use of non-chemical intervention methods. Negative air ionization is known to be a promising technology that is a safe and non-toxic means of eliminating dust and pathogens from the air. The Electrostatic Space Charge System (ESCS) has been shown to reduce airborne levels of *S.* Enteritidis by 95% in caged-layer rooms (Holt *et al.*, 1999) and to reduce airborne transmission by 98% in controlled-environment cabinets (Gast *et al.*, 1999). These benefits were attributed primarily to the reduction in levels of airborne dust (Mitchell and King, 1994; Mitchell, 1998). It is well known that most airborne bacteria are attached to dust particles (Al-Dagal and Fung, 1990). The process by which the ESCS can remove air-polluting microorganisms in the vicinity of poultry involves charging airborne particles and collecting them on special collector plates located on the floor or the walls. Ionization studies with *S.* Enteritidis suggest that a significant step towards producing eggs that are free from this organism can be achieved by improving air quality in houses where the eggs are produced (Holt *et al.*, 1999). This concept has been shown to work in filtered-air, positive-pressure (FAPP) houses used to produce disease-free poultry (Furuta *et al.*, 1976). Air ionization can kill airborne and surface-associated microorganisms.

Initial work on the effects of the ESCS on biofilms shows that this approach has promise as a viable means of reducing bacterial contamination on surfaces. Natural bacterial populations were collected from a poultry processing facility, allowed to multiply and form biofilms, and assessed for susceptibility to negative air ionization. A small chamber with an ESCS was used to treat the mixed bacterial populations growing on small stainless steel coupons. The object of the system was to transfer a strong, negative electrostatic charge to inoculated coupons located at the base of the chamber. The system effectively decreased levels of bacteria on the stainless steel by 99.8%. These results demonstrate the potential efficacy of negative air ionization against surface contamination in the poultry processing environment (Arnold and Mitchell, 2002).

The reason for using the ESCS is to reduce developing or pre-existing biofilms by transferring a strong, negative electrostatic charge to bacterial cells within biofilms growing on stainless steel surfaces. The ESCS dramatically reduced bacterial levels in every trial. Treatment of *Campylobacter jejuni, E. coli*, *S.* Enteritidis, *L. monocytogenes* and *Staph. aureus* achieved up to a 4-log reduction within three hours (Arnold *et al.*, 2004). Both Gram-positive (*L. monocytogenes*, *Staph. aureus*) and Gram-negative (*C. jejuni*, *E. coli, S.*

Enteritidis) organisms were tested to determine whether structural differences in the cell wall would affect killing. For the five species tested, the system was effective against both types of bacteria, but there were differences between species in the degree of kill obtained. The most sensitive were *L. monocytogenes* and *S.* Enteritidis. These results support the conclusion of a previous study (Arnold and Mitchell, 2002) that the effect of the ESCS varies according to species. Other important physiological factors may be determined with further research.

The use of spores of *Bacillus anthracis* (causative agent of anthrax) as a possible weapon for bioterrorism has focussed attention on the need for new methods of detecting and killing bacterial spores. Generally, bacterial spores are extremely resistant to processes that kill vegetative cells and sanitizing agents usually have little or no effect (Yan *et al.*, 1992; Sigmond *et al.*, 1999). Treatment of spores of *B. stearothermophilus* with the ESCS achieved up to a 3-log reduction within three hours (Arnold *et al.*, 2004). Together with the above-mentioned effects on vegetative cells of pathogens, this suggests that the ESCS is a promising alternative treatment for reducing the microbial load in food-processing facilities and has the potential to reduce the usage of antimicrobials. The economic feasibility of using this technology in a poultry processing facility remains to be determined, but, if shown to be effective on a large scale, the basic equipment cost would probably be comparable to that of existing disinfection equipment and chemicals.

15.4 Sanitation operations

Controlling bacterial pathogens is necessary for the production of safe food. Bacteria thrive over a wide range of temperatures and especially in wet environments, such as those of meat and poultry processing plants. They can be carried in condensation and process water, on boot soles, brushes and brooms, as well as on a variety of equipment and the hands of operatives. Multiple strategies and constant monitoring are required to ensure control.

15.4.1 Processing practices

Some intervention strategies in the processing plant are aimed at the removal of bacteria before they can become a problem. For example, ingesta and feces of animals contain many bacteria and, typically, feed withdrawal for 8–12 hours before slaughter is used to reduce the numbers of bacteria in the intestines of incoming birds. Flow patterns in the processing plant are designed so that contamination with any pathogens that occur can be reduced while the product moves through the plant. Carcasses travel from dirty to clean areas, air moves from clean to dirty areas and people move from clean to dirty areas during clean-up.

Scalding areas can include a pre-scald stage with brush machines or water sprays that can include 20 ppm chlorine to help remove fecal matter and bacteria.

Scalding may be of the multi-stage, counter-flow type. In this, carcasses are moved to cleaner water in each successive tank. Scalding reduces levels of carcass bacteria by about 10,000 per carcass, but not much change occurs in the numbers of *Salmonella* present. Spray-scalders are thought to minimize the potential for cross-contamination, but a 'hard' scald can remove feathers and skin, and damage the product. Automated re-hang that transfers carcasses by machine between the defeathering and evisceration lines serves to reduce surface cross-contamination from worker handling. The evisceration line removes the viscera from the carcasses. Both spoon eviscerators (leave viscera on carcasses) and the gripper type (leaves a pack next to each carcass) allow dripping during and after inspection of the viscera. The carcasses progress to inside/outside carcass washers that can include chemical sprays. The final chilling process can be by water immersion or air-blast. Immersion chilling can use the counter-current method and chemicals, such as chlorine or ozone, to remove and kill bacteria. Air chilling can include a water mist with chemicals added.

15.4.2 Cleaning and monitoring

Processors may do light cleaning throughout the working day. However, most operators conduct a full-blown cleaning and sanitizing program, and apply longer-acting products, such as fogs and foams, after hours and at weekends. The common steps in a plant sanitation program begin by pre-rinsing equipment and surfaces with a high-pressure water spray, followed by washing or scrubbing with a chemical application (Arnold, 2003). Detergents may be used to wet, emulsify, lift and suspend soil for removal. Disinfectants may be used to reduce or inhibit growth, destroy bacterial cells, but not necessarily spores or viruses. Foams and fogs give increased chemical activity due to longer contact time, can lower levels of detergent use, penetrate hard-to-reach areas and allow easy rinsing. Applications of specific chemicals for particular problems or areas of the processing plant are shown in Section 15.3.4. Training plant personnel in the application procedures is critical to achieve the necessary concentration, pH, contact time and temperature for optimum efficacy of cleaning products. The washing steps are followed by pre-operation inspection before a final rinsing and santitizing. Sanitizers are usually no-rinse and non-foaming, and kill a broad spectrum of microbes. Deep cleaning requires the removal of outer panels, guards, covers and other mechanisms to reach innermost seams and joints that can trap meat particles. However, dismantling equipment for deep cleaning can incur excessive downtime. Steam cleaning with wands can heat hard-to-reach areas to temperatures that are lethal to microbes (*Meat Processing*, 2004).

Methods based on bacterial adenosine triphosphate (ATP) bioluminescence and conventional microbiological plating methods are most often used to monitor the effectiveness of cleaning and disinfection operations. Several new biotechniques are employed by microbiologists to conduct surveys of plant environments and processing equipment and detect any hot-spots of specific pathogen contamination. These include electrical methods, such as impedance/

conductance methods, the direct epifluorescent filter technique (DEFT), flow cytometry, biosensors, agglutination or other immunological tests and nucleic acid technologies, such as the polymerase chain reaction (PCR), plus ribotyping and use of microarrays (Anand and Griffiths, 2004). Improvements in these technologies move towards real-time measurements, with which the industry can obtain a result in minutes rather than in hours or days. The difficulty lies in the detection of bacterial cells buried in a food matrix.

15.5 Assessment of effectiveness

Reducing bacterial contamination of poultry products is a major concern among processors and those interested in food safety, because chicken, turkey and egg products are frequently incriminated in outbreaks of foodborne illness (Gibbs *et al.*, 1978; Adams and Mead, 1983; Franco *et al.*, 1995; Smith and Fratamico, 1995). Mechanical equipment has vastly increased the number of carcasses that can be processed by a single plant in a day. However, the addition of equipment to increase automation has resulted in the presentation of new surface areas for carcasses to contact repeatedly and, thus, new opportunities for bacterial attachment and cross-contamination (Hinton *et al.*, 2003; McEldowney and Fletcher, 1988).

Effective tracking and monitoring systems ensure that quality control criteria are met consistently, but there is disagreement about the frequency and necessity for testing, while lack of uniformity in the methods used is common. Many disparate assays purported to detect the presence and numbers of pathogens give conflicting results and are time-consuming and expensive, or lack sensitivity. Another deterrent to obtaining good information on the significance of bacterial contamination and the behavior of pathogens is the lack of appropriate models that simulate the processing environment. Smaller, pilot versions at academic and government research institutions attempt to mimic the industrial processing operation. While they can give some information about specific equipment and the spread of contamination, they are inadequate to imitate properly the problems caused by the sheer volume and size of a real plant in operation (Arnold, 1998).

15.5.1 Cost to the industry

It is in the best interests of the food industry to produce safe, high-quality food products that people will buy. Employee training is essential and workers need to know why their movements must be controlled. For example, employees have been seen stepping around footbaths, because using the bath takes time (Bjerklie, 2003). Employees need to know why sanitized footwear, gloves, hairnets and other safeguards are important in relation to product hygiene.

HACCP raises costs. The fraction of costs for the Pathogen Reduction (PR/HACCP) rule of 1996 was about the same for large as for small plants, suggesting that larger plants were no better able than small plants to absorb

sanitation and process-control costs. Data also suggest that PR/HACCP raised wholesale meat and poultry prices by about one per cent (Ollinger and Mueller, 2003).

15.5.2 Impact on food safety

Customers do not expect to contract a foodborne illness from products they consume and may severely punish a plant that fails to provide wholesome food. For example, Hudson Meats had to sell its hamburger operations after one of its plants was found to have produced hamburgers contaminated with *E. coli* O157:H7. Also, Sara Lee lost hundreds of millions of dollars when it was identified as the source of products contaminated with *L. monocytogenes* that killed several people (Perl, 2000). Lack of a sufficient sanitation program can be a significant factor in the demise of a company (Ollinger and Mueller, 2003).

15.6 Future trends

The very rapid globalization of the food trade increases the probability of the global spread of foodborne illnesses. It is certain that new pathogenic bacteria and viruses will appear on a regular basis, and there is a need to be able to react quickly. Global food safety regulations, based on scientific data, will be needed to ensure safe exchange of products. Cooperation to provide this base must come from industry, trade associations, academia and government.

Every operational sanitation program must support HACCP requirements, achieve fast screening of test samples, reduce inventory holding times and achieve rapid intervention. Information technology in the form of software and data analysis systems can improve the HACCP approach. In future, monitoring can be automated, making it online, using scanners. Microbial test devices can transfer data to processors to allow the analysis of historical trends (Sperber, 2004), giving faster response times. The product must be safe when it leaves the processing plant.

The use of innovative techniques to reduce bacterial contamination during processing of poultry has the potential to improve product quality. Food safety could also be improved by increasing the use of materials that are resistant to growth and attachment of microorganisms, while decreasing the use of materials that are conducive to these phenomona. Preventing the build-up of bacteria and food debris into biofilms during processing will expedite the efficient use of sanitizers.

Developing a model that represents such a complex phenomenon as biofilm formation and devising quantitative tests that can predict the behavior of living organisms within it, have been long-standing goals. Before this can be done, improvements are needed in bacterial sampling, identification and enumeration, especially for pathogens. The relative importance of each species and their interactions in the initiation, build-up and longevity of a biofilm is unknown.

Identifying the role that pathogens play in bacterial attachment and biofilm formation is a necessary step towards a better knowledge of pathogen persistence in processing plants. Understanding these processes and use of more appropriate finishes for equipment surfaces will assist in developing methods to prevent bacterial attachment and biofilm formation, which will ultimately improve sanitation practices and pathogen control. Reducing the present use of chemicals for plant sanitation will lower consumer costs and the negative effects of agriculture on the environment.

15.7 Sources of further information and advice

15.7.1 References to consult

1. Chemistry of disinfectants and disinfectant by-products, analytical methods, mechanisms involved in the formation of by-products, influence of source-water on the amounts and types of by-products formed (Amy *et al.*, 2000).
2. Environmental health criteria for disinfectants and disinfectant by-products, kinetics and metabolism in laboratory animals and humans, toxicology of disinfectants and disinfectant by-products, epidemiological and risk studies (Amy *et al.*, 2000).
3. Effects of sanitation and process-control costs for implementing rules and regulations on meat and poultry producers. Promulgation of the Pathogen Reduction/Hazard Analysis and Critical Control Point rule of 1996. Effects of controls on long-run profits, with plant survival as a measure of profitability. (Ollinger and Mueller, 2003).
4. Development of bacterial biofilms during poultry processing, sanitation steps for processing plant surfaces, table of chemicals for control of microbial growth and biofilm formation (Arnold, 1998, 2003).
5. The history, manufacture, properties, hazards and uses of chlorine; super-chlorination, chemistry of chlorination, chlorination of wastewater, operation and maintenance of chlorination and dechlorination equipment, chlorine dioxide, ozone, peroxone, bromine and iodine, useful charts for computing dosages (White, 1999).

15.7.2 Industry and interest groups, websites

1. Biofilms Online – news, features, trade information and applied research on microbial biofilms. http://www.biofilmsonline.com
2. EU Poultry Industry – analysis of the future role of the EU industry in a global setting www.wattnet.com/Archives/Docs/900pi67.pdf?CFID=25710&CFTOKEN=74030876
3. Poultry Science Association – Association news, journal contents, jobs, meeting information and related items. http://www.poultryscience.org
4. The Coop – focused on the small producer of poultry, breeding stock and eggs for limited distribution or family consumption. The major goal of the

site is to act as a focus for non-commercial and general-interest poultry information, educating the public and those involved in small-scale poultry production. http://www.the-coop.org

5. Food Safety and Inspection Service – the regulatory agency in the US Department of Agriculture responsible for ensuring that the commercial supply of meat, poultry and egg products is safe, wholesome and correctly labeled and packaged, as required by the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act. http://www.fsis.usda.gov
6. US Poultry and Egg Association – industry organization, sponsor of the world's largest poultry trade show. http://www.poultryegg.org/ScientificForum/index.html

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16

HACCP in poultry processing

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16.1 Introduction

The Hazard Analysis Critical Control Point (HACCP) system is used as a systematic way of identifying potential public health hazards in the food industry, assessing those hazards and ultimately controlling them. By considering the overall production process, the HACCP system allows companies to prevent hazards from occurring in the first place, instead of relying on end-product testing to determine whether the hazards have already occurred.

The HACCP concept was first developed in the 1960s by the US National Aeronautical and Space Administration and the Pillsbury Corporation to ensure that astronauts would not contract foodborne illness while in space. The system began to be applied in the food industry after the National Academy of Sciences recommended its use in a 1985 report: 'An evaluation of the role of microbial criteria for foods and food ingredients.' The US Food Safety and Inspection Service (FSIS) took the HACCP system to a new level by making it a part of their regulatory program. Thus, poultry processors in the USA were required to have HACCP plans. The HACCP plans formulated by each plant then become the regulations under which that plant operates. This chapter will cover the principles of the HACCP system, as described by the National Advisory Committee on Microbiological Criteria for Foods[1] and then discuss briefly HACCP plan development, implementation and enforcement. The chapter will begin with the process of writing a HACCP plan to help the reader understand some of the key components of each step in plan development. Once accepted by the FSIS, the plan must be implemented by the processor. Therefore, careful consideration must be given to developing, applying and re-assessing the plan in relation to its effectiveness.

16.2 Preliminary tasks

Before writing the actual HACCP plan, the following preliminary tasks should be completed. Firstly, copies of all prerequisite programs (see below) and a sample of data collected from the programs should be assembled for reference use. Also, FSIS has moved away from prescriptive sanitation procedures to using sanitation performance standards. This move has placed more responsibility on the establishment to determine how they can meet a specific standard. However, there are certain sanitation records that a prudent establishment will have. They include:

- information on pesticide usage
- the approval letter for sewage disposal, when using a private system
- water potability certificate
- chemical material safety data sheets.[2]

The Federal Meat Inspection Act and the Poultry Products Inspection Act provide authority, requirements, policies and standards relating to plant sanitation. FSIS requirements for sanitation are found in Part 307, sections 307.1-3, Part 381, subpart G and Part 416. Specific instructions on how FSIS verifies sanitation in an establishment can be found in FSIS Directive 5001.1, Revision 1.[2]

Before the process of developing the HACCP plan begins, a complete review of current prerequisite programs is required. These cover specific procedures, including good manufacturing practices that address operational conditions and provide the foundation for the HACCP system. Common examples are those that address maintenance schedules, ingredient specifications, sanitation standard operating procedures (SSOPs), *Listeria* control programs and employee training. Only documented programs that relate to product safety may be used to support the HACCP plan. In addition, the data collected from prerequisite programs should be reviewed to assess their role as a part of an overall food safety program. A deviation in the results expected from a prerequisite program can cause a corresponding deviation in the HACCP plan. Therefore, if any changes are made in prerequisite programs, a reassessment of the HACCP plan must be made to ensure that any new hazards are being addressed.

One benefit from this close review of existing programs is an evaluation of the impact that one program may have on another. Depending on the programs in force, it may be possible to reduce the total number of Critical Control Points (CCPs) in the final HACCP plan. For example, a prerequisite program that includes the monitoring of temperatures throughout the plant could remove the necessity for a CCP that controls refrigeration temperature in a food-storage area. Another example, commonly found in cooking plants, is a separate *Listeria* control program rather than a specific CCP to control *Listeria* in the ready-to-eat product.

16.3 Assembling the HACCP team

A multidisciplinary team of people should be assembled to work on the HACCP plan. The team should include people with experience in engineering,

production, plant sanitation, quality assurance, plant security and food microbiology. It is imperative that the team has a leader who will take the responsibility of seeing that the plan is implemented, reviewed and revised, as needed. Thus, the responsibilities go beyond just writing the plan. The team members, too, need to be actively involved in implementing and re-assessing the plan. As members leave the team over time, new members need to be appointed. It is crucial that the diversity of expertise in the team remains as broad as possible and relevant to the task in hand.

16.4 Describing the food and its distribution

Once the team is assembled, it should elaborate a general description for each of the items produced in the plant, encompassing their ingredients and how they are processed, as well as the conditions under which they are finally distributed, including temperature. As new products are developed, they need to undergo the same hazard analysis as those done previously. If new hazards are identified, the HACCP plan will need to be modified.

16.5 The intended use and consumption of the food

It is important to know whether a particular subset of the population, such as infants, the elderly or immunocompromised individuals, is among the intended consumers of the product being evaluated. If the product is intended specifically for more susceptible individuals, additional safety steps may need to be incorporated in the production process.

16.6 Developing a flow diagram that describes the process

A simple flow diagram of the process, showing all inputs and outputs, should be developed. This diagram should be a clear, simple outline of all the steps that the establishment controls. It is also helpful to have a schematic representation of product flow through the plant. The flow diagram should be checked by comparing it to the actual process occurring in the plant. All ingredient and product additions and removals must be accounted for in the flow diagram, but it is important to keep each step of the process separate. This flow diagram will be utilized in the hazard analysis for the HACCP plan. It becomes very difficult to conduct a hazard analysis when different steps have been combined in the flow diagram. Whenever new equipment is installed, or modifications are made to current equipment or processes, a new hazard analysis must be conducted to ensure that no new hazards have arisen, due to the changes made in either the equipment or the process. A change in line-speed is a good example of a change that should trigger a hazard re-evaluation, but this is often overlooked.

16.7 HACCP principles

The seven HACCP principles are:

1. Conduct a hazard analysis.
2. Determine CCPs.
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions.
6. Establish verification and validation procedures.
7. Establish record-keeping procedures.

16.7.1 Conducting a hazard analysis

The purpose of the hazard analysis is to develop a list of significant hazards that are reasonably likely to cause harm, if not controlled. Physical, chemical and microbial hazards (covering aspects such as product ingredients, characteristics, processing procedures, plant design, equipment, food security, microbial content) should all be considered. The next step is to decide on the hazards that need to be addressed in the HACCP plan. This is done by looking at the severity of each hazard (and what would happen if the hazard is not controlled), the likelihood of the hazard occurring, whether prerequisite programs are in place to control the hazard, etc. Once the hazard evaluation has been made, then the hazards arising at each step of the process are listed, along with any means of controlling them. For example, at the cooking step, *Salmonella* is a potential hazard, because it has been associated with outbreaks of foodborne illness from undercooked poultry. The hazard should be addressed in the plan and it can be prevented by adequate cooking in the factory. This kind of information is often recorded in a table.[1]

The HACCP regulations require that each establishment maintains certain records that document the HACCP plans (9 CFR 417.5). These records include the written hazard analysis prescribed by 9 CFR 417.2 (a), supporting documentation and any other related materials. An establishment may conduct certain testing or monitoring activities as part of the HACCP plan, or in programs that could affect the hazard analysis, but may or may not be referenced in the HACCP plan. For example, these activities may be part of a prerequisite program or a program of product testing carried out to comply with the specifications of business customers. Because the results of such activities could affect the hazard analysis, FSIS considers the records that document the results of any monitoring or testing carried out to be supporting documentation for the hazard analysis. Under 9 CFR 417.5, the records must be maintained by the establishment and made available for review by FSIS. Furthermore, these records must be kept for the same length of time as the HACCP records, since they are viewed as supporting documentation.[3]

Misidentification of a hazard is a common error in hazard analysis. For example, temperature is often identified as a hazard, whereas it is actually the preventative

measure. Instead, the particular microorganism that needs to be controlled is the hazard and the more specific its identification (e.g. biological, microbial hazard, pathogen, *Salmonella*), the better the opportunity to control it. For example, if 'pathogens' are given as the hazard, any attempt to control temperature as the preventative measure is too vague. Temperature control could be a preventative or control measure for *Salmonella*, but it would not be appropriate for *Listeria*, because this organism can grow well at refrigeration temperatures.

16.7.2 Determining critical control points

A CCP is a step at which a control measure is used and it is essential to prevent, eliminate or reduce a hazard to an acceptable level. Examples of CCPs include thermal processing, chilling, testing products for chemical residues and using a metal detector. A 'CCP decision tree' (Fig. 16.1) is often used in establishing whether or not a step should be considered a CCP. Questions asked[1] include:

- Does this step involve a hazard of sufficient severity and frequency to warrant its control?
- Does a control measure for the hazard exist at this step?
- Is control at this step necessary for product safety?
- Is control at this step necessary to prevent, eliminate or reduce the risk of the hazard to consumers?

If the answer to each of these questions is yes, then the step is probably a CCP. FSIS does not indicate where CCPs should be located. The exact locations are based on the evaluation of hazards carried out at the plant and the determination of appropriate control measures. However, FSIS does require that all slaughter plants have a CCP to address visible fecal contamination and ensure that no fecal material enters the chiller. For plants producing ready-to-eat products, FSIS requires some kind of *Listeria* control, either as a CCP or a separate control program.

If a plant has a history of *Salmonella* failures, sanitation non-compliance records (NRs) etc., it is difficult to provide convincing support for the claim that the hazard is under control and a CCP is not needed. In addition, if a plant has to recall a product because of a safety hazard, the root cause of the problem must be determined. When this has been done, the HACCP plan must be re-assessed to establish whether the recall was due to failure of the plan or the occurrence of an unforeseen hazard. In either case, the plan must be adjusted to ensure that the same problem does not happen again.

One of the most common CCPs in primary processing is that directed at controlling the growth of *Salmonella*. This is usually located at the chilling stage.

16.7.3 Establish critical limits

This step requires that critical limits are set for each CCP. A critical limit is the maximum or minimum value for a measure that is required to control a hazard. Critical limits are often based on temperature, time, physical dimensions,

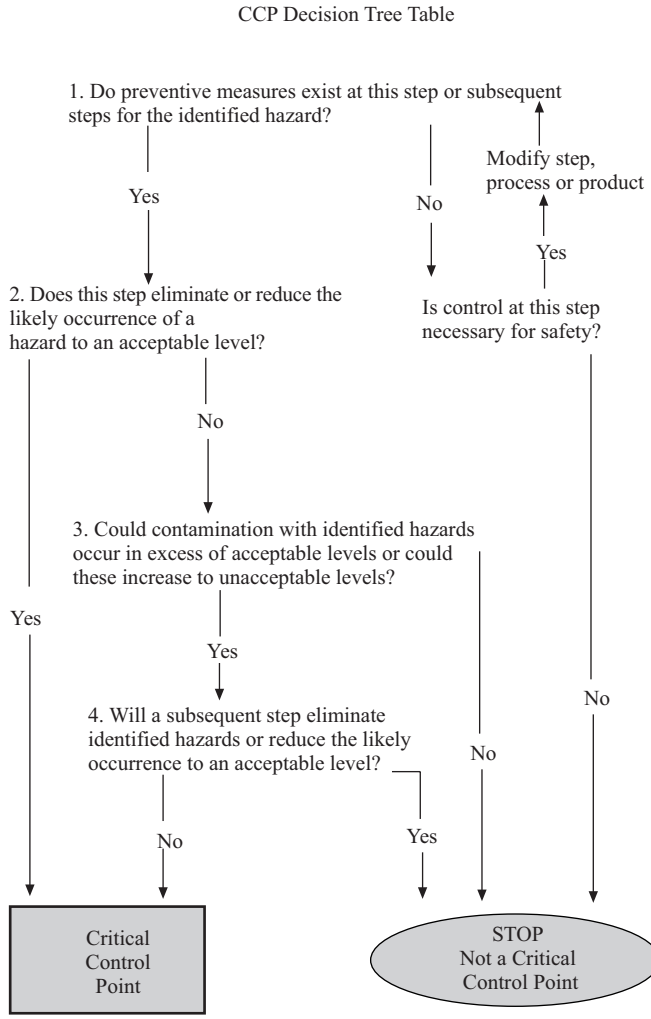

Fig. 16.1 Decision tree adapted from NACMCF.[1]

humidity, moisture content, water activity, pH, titratable acidity, salt concentration, available chlorine, viscosity, use of preservatives or sensory information. They may be obtained from regulatory standards, literature surveys, experimental results and expert opinion. An example of a critical limit in carcass chilling is to require the deep breast muscle to be cooled to no more than 40 ºF.[1]

Critical limits are often based on factors such as performance standards, safe harbors, current or former regulations, or scientific research. Regardless of the basis, written documentation must be on file to support the limit chosen. If the limit is based on a directive, a copy of that directive should be kept on file as part of the validation for the HACCP plan.

Many plants use indirect measurements to provide critical limits. For example, chill-water temperature is often monitored instead of carcass temperature. The regulations provide a given amount of time for a carcass to reach 40 ºF, based on the weight of the carcass. If a plant sets a temperature of 40 ºF for the exit of the chiller (the point most often selected), it is actually creating a regulation under which the plant must operate that is more exacting than any applied in other establishments. However, if the plant elects to monitor the temperature of the chill-water rather than that of the carcass, there must be data showing that the normal time-period for carcass chilling is sufficient to achieve the 40 ºF limit set by the regulations. Obtaining this information is part of the validation process and the information would need to be kept in order to support the critical limit.

16.7.4 Establishing monitoring procedures

These procedures are needed to determine whether or not a process is under control. Trends leading to loss of control are also identified through monitoring. Typical monitoring procedures include checking temperatures, pH values and moisture levels. Monitoring procedures are most helpful when they are rapid and easy to carry out. This allows the processor to take immediate action if a critical limit is exceeded or not met. An establishment must be able to provide a justification for the sampling method, size, time, etc. Some plants have used sampling approaches (size, time, procedures) that were utilized previously by FSIS. In such situations, a copy of the supporting documents (directive, etc.) should be on file, as a part of the validation for the HACCP plan. In other situations, where a complete plan is not available, any supporting documents used to determine the necessary monitoring procedures should also be a part of the validation file.

It is very important that monitoring procedures address what is being monitored, who should do the monitoring, how it should be done and when it should be carried out. The people involved in conducting the monitoring procedures (usually the primary monitor and any potential substitutes) *must* be trained properly. They need to realize the importance of their particular responsibility and to understand that, if they miss a monitoring occasion, it can be very difficult to prove that the safety of the product has not been compromised. The monitor also needs to know what action should be taken if a deviation occurs. When selecting the appropriate frequency of monitoring, consideration should be given to balancing the practicalities (time, cost, etc.) with the frequency needed to identify a process that may be moving towards a deviation *before* that deviation actually occurs.

16.7.5 Establishing corrective actions

Whenever there is a deviation from a critical limit, corrective action must be taken to ensure that a potentially unsafe product is not released. When potential deviations can be predicted, reasons for such deviations, along with the corrective action needed to ensure that the product will be safe, should be

recorded for each CCP. When any corrective action is undertaken, a written record should address the cause and correction of the non-compliance, the safety of the non-compliant product and the nature of the action that was taken. A deviation does not mean that the HACCP plan is not working, unless it occurs on a regular basis and is not detected and corrected. However, if a review of the records shows that the same corrective action is being taken regularly, then the HACCP plan should be re-assessed to determine whether there is a better way to control the hazard, so that frequent deviations do not occur.

When taking corrective action, documentation is critical! This should include the identification of the problem, how the problem was corrected, the disposition of the product and how the deviation can be prevented in future. If an opinion is obtained from an outside expert on the disposition of the product, a written copy of that advice must be retained as a part of the documentation for the corrective action. When completing the documentation, the root cause of the problem must be addressed. If a deviation continues to occur, FSIS may consider these incidents when linking NRs.

16.7.6 Establishing verification procedures and validation of the HACCP plan

Verification is the process of ensuring that the HACCP plan is working as intended. The plan should be verified as soon as it is implemented and then at set times, such as yearly, or whenever it is suspected that the plan is inadequate. A review of the records is a common practice in verification. The review should aim to identify trends and verify that all critical limits are being met, while ensuring that records are complete prior to pre-shipment sign-off, etc. In addition, calibration of any measuring equipment such as thermometers, is a part of verification. It is recommended that plants conduct their thermometer calibrations with FSIS inspection personnel.

Validation ensures that the HACCP plan is capable of achieving the objective of reducing product contamination with pathogens. As discussed earlier, supporting documentation for the choices made in developing the plan are a part of validation. The documentation may include research studies showing that chilling the product to a specific temperature reduces microbial growth, or a safe harbor temperature that has been accepted historically by FSIS. Supporting documentation for any decisions that have been made throughout the development of the HACCP plan is necessary for validation. It is the responsibility of FSIS to verify that the establishment has met all the requirements.

FSIS is now looking more critically at the validation of HACCP plans. Therefore, establishments should consider their entire food safety program (HACCP plans, prerequisite programs, etc.) during the validation process. This should include the ways in which the various programs are linked. Often, supporting programs are changed without much thought being given to the impact of the changes on other programs.

16.7.7 Establishing record-keeping and documentation procedures

Documentation is extremely important when using a HACCP plan. The only way to prove that critical limits have been met is by examining the records on processing and monitoring. Documentation on the preliminary tasks and application of each HACCP principle should be kept, as well as all records of monitoring and verification.

A review of the records is useful in determining trends and can help to maximize product safety. For example, when reviewing records, it might become clear that birds from Farm X are consistently dirtier (with higher microbial counts) than birds received from other farms. Birds from Farm X could be processed last in the day to reduce contamination of equipment, thereby, reducing cross-contamination of carcasses coming in from other sources. Another example could be a review of records associated with fecal contamination. The review should cover HACCP records and any other associated documents (records of prerequisite programs, NRs, etc.). If the trend in fecal contamination is on the rise, it could indicate a need for better maintenance of evisceration equipment, and such maintenance can be carried out before a deviation occurs.

16.8 Enforcement

FSIS looks beyond the written HACCP document to determine the adequacy of the plant's HACCP system. All available evidence and supporting documentation are considered. FSIS also evaluates other systems within the plant, such as SSOPs, in-plant testing programs, environmental controls, end-product testing etc.

The *Salmonella* performance standards were designed by FSIS to verify the adequacy of HACCP systems. Data were used from the nationwide baseline studies to establish performance standards for different categories and classes of poultry. FSIS then used data collected by routine testing, after implementation of HACCP and other food safety systems, to verify the adequacy of control systems for individual establishments. Reliance is placed on the National Advisory Committee on Microbiological Criteria for Foods for scientific guidance.

The pathogen-reduction performance standard applies to separate establishments, not to individual products. The latter are not tested to determine their disposition, but rather to measure the effectiveness of the slaughter or meat-grinding processes used in limiting *Salmonella* contamination. Establishments do not have to hold or recall a product on the basis of the results obtained for *Salmonella*. The performance standards are regulatory requirements. Samples are taken in sets and the results of an entire set are used to determine whether or not an establishment is meeting the performance standards. The *Salmonella* performance standards for raw meat and poultry, and for raw, ground products are given in Code of Federal Regulations, Title 9, Chapter III,

Part 310.25 and Part 381.94. The goal of the *Salmonella* testing program is to protect the consumer from contaminated products, especially from fecal contamination, by verifying that the performance of each establishment meets the *Salmonella* standards.[4] FSIS also uses the *Salmonella* performance standard to measure the effectiveness of the HACCP plan. Therefore, if the establishment fails to meet the performance standard, the HACCP plan must be re-assessed. In many cases, a Consumer Safety Officer (CSO) will conduct a comprehensive assessment of all food safety programs in an establishment that has failed the *Salmonella* performance standard. The CSO is not one of the inspectors located in the plant, but rather a regional officer with specialized training in food safety. The CSO conducts a comprehensive assessment of each individual food safety program and determines any design flaws. All of the food safety-related programs at a given establishment should interact with one another to provide a sanitary environment for the production of a safe food product. The CSO does not concentrate on any individual aspect, but bases the assessment on how well the whole system functions.

Directive 5000.2 instructs inspection personnel to seek access to the data that is generated by any monitoring or testing that is related to food safety, in order to ensure that this data is available when inspection personnel are verifying HACCP records. Directive 5000.2 shows clearly that inspection personnel should review any food safety data that has been generated by the establishment at the weekly HACCP meeting. Establishments may conduct certain testing or monitoring activities as part of their HACCP plan, or in programs that could affect the hazard analysis, but may or may not be referenced in the HACCP plan. For example, establishments may perform testing or monitoring activities as a part of a prerequisite program or conduct product testing to comply with customer specifications. Because the results of such testing and monitoring could affect the hazard analysis, FSIS considers the relevant records to be supporting documentation for the hazard analysis, which, under 9 CFR 417.5, must be maintained by the establishment and made available for FSIS to review. For this reason, these records must be maintained for the same length of time as the establishment's HACCP records.[3]

A HACCP non-compliance is the failure to meet any of the regulatory requirements of §417 (monitoring, verification, record-keeping, re-assessment or corrective action). If the plant finds the cause of the non-compliance, takes appropriate corrective action and applies preventative measures, there is no longer a non-compliance. If the plant does not take immediate action and has further planned action to control the problem, FSIS will document it as a NR. Should FSIS discover the non-compliance, while the plant did not, then FSIS issues a NR. If a HACCP non-compliance occurs and is notified, the plant is expected to document immediate and further planned action to correct the non-compliance.[5]

FSIS monitors trends in non-compliance and will link similar NRs. Linkage is necessary to support further enforcement action. FSIS inspectors use their judgment when determining which NRs to link together. They must decide

whether a second NR is an isolated incident or represents a developing trend. Questions typically asked in making the judgment are:

- How much time has elapsed since the previous NR was issued?
- Was this non-compliance from the same root cause as the previous one?
- Were the plant's further planned actions implemented?
- Were the plant's further planned actions effective in reducing the frequency of these non-compliances?
- Is the plant continuing to implement better, further planned actions?[5]

If a NR is linked to a previous one, the linkage will be documented on the current NR. These NRs should be linked as the non-compliance occurs. Several NRs should not be linked without due consideration. The Rules of Practice in 9 CFR 500 provide plants with due process. They also lay out how FSIS progresses further enforcement actions and under what circumstances. [5]

16.9 Imports and exports

While foreign regulatory systems for foods need not be identical to the US system, they must employ equivalent sanitary measures and provide the same level of protection against food hazards as that achieved domestically in the USA. Meat, poultry and egg products entering the USA must bear labeling that meets the same FSIS requirements as those for the corresponding domestic products. All labels bearing special claims require an evaluation before they can be distributed in commerce.[6]

For most countries, all USDA federally-authorized meat, poultry and egg-product establishments are eligible to export, provided that the requirements of the receiving country are met. Some countries, including those of the European Union, require US establishments to be approved, registered and/or reviewed prior to exporting their goods. Such countries may periodically audit the establishments exporting to them. To assist US exporters, the USDA Foreign Agricultural Service (FAS) provides a website (*http://www.fas.usda.gov/*) containing FAS contacts at embassies located throughout the world, as well as the Ag Exporter Assistance, which contains helpful information on exporting from the USA.[7]

16.10 Future trends

Each year, FSIS continues to look more critically at HACCP plans in food establishments. New matters of emphasis or a more critical review of the plans and the addition of new performance standards are a possibility. Establishments should think of their food safety programs as living documents and have a mechanism for their regular re-assessment and modification.

16.11 Sources of further information and advice

There are a lot of HACCP-related materials available online. From the online resources, the most up-to-date information is available for review. The FSIS website is an excellent source of HACCP-related topics. The following is a list of some of the most useful sources.

FSIS Guidebook for Preparation of HACCP Plans and Generic Models. Available online at: http://www.fsis.usda.gov/Science/Generic_HACCP_Models/index.asp (Accessed June 21, 2004).

FSIS Safety and Security Guidelines for the Transportation and Distribution of Meat, Poultry and Egg Products. Available online at: http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/Oa/topics/transportationguide.htm (Accessed June 16, 2004).

Acts and authorizing statutes

Poultry Products Inspection Act. Available online at: http://www.fsis.usda.gov/regulations_&_policies/Poultry_Products_Inspection_Act/index.asp (Accessed June 16, 2004).

Code of Federal Regulations (CFR)

9 CFR 381 Poultry Products Inspection Regulations. Available online at: http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/index_archive.htm (Accessed June 15, 2004).

9 CFR 416 Sanitation Requirements. Available online at: http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/index_archive.htm (Accessed June 16, 2004).

9 CFR 417 Hazard Analysis and Critical Control Point Systems. Available online at: http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/index_archive.htm (Accessed June 15, 2004).

FSIS directives

5001.1, Revision 1, Verifying an Establishment's Food Safety System. Available online at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/5000.1Rev1.pdf (Accessed June 16, 2004).

5000.2 Review of an Establishment Data by Inspection Personnel. Available online at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/5000.2.pdf (Accessed June 16, 2004).

5500.1 Conducting Targeted In-Depth Verification Reviews. Available online at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/5500.1.pdf (Accessed June 16, 2004).

Import and export information

Export information can be found online at: http://www.fsis.usda.gov/regulations_&_policies/Poultry_Products_Inspection_Act/index.asp (Accessed June 16, 2004).

Import information can be found online line at: http://www.fsis.usda.gov/Regulations_&_Policies/Import_Information/index.asp (Accessed June 16, 2004).

16.12 References

1. NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS (1998), 'Hazard analysis and critical control point principles and application guidelines', *Journal of Food Protection*, 61, 1246–1259.
2. FSIS (2004), *Food Safety Regulatory Essentials HACCP-Sanitation*, Washington, DC, US Department of Agriculture.
3. *Federal Register*, Volume 69, Number 86, May 4, 2004, page 24557.
4. FSIS (2004), *Food Safety Regulatory Essentials HACCP-Raw*, Washington, DC, US Department of Agriculture.
5. FSIS (2004), *Food Safety Regulatory Essentials HACCP-NRTE/RTE*, Washington, DC, US Department of Agriculture.
6. *FSIS Regulatory and Policy. Import Information.* Available online at: http://www.fsis.usda.gov/Regulations_&_Policies/Import_Information/index.asp (Accessed June 16, 2004).
7. *FSIS Regulatory and Policy. Export Information.* Available online at: http://www.fsis.usda.gov/Regulations_&_Policies/Export_Information/index.asp (Accessed June 16, 2004).

17

On-line physical methods for decontaminating poultry meat

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17.1 Introduction

There is a variety of approaches to reducing the incidence of pathogens in retail poultry, and these can be targeted at various control points, from 'farm to fork'. Since pathogens present on the live birds can still be detected on carcasses after processing (Newell *et al*., 2001), it is clearly desirable that birds delivered to the processing plant should have low levels of contamination, and it is logical to target primary intervention at the poultry farm. However, methods to improve the biosecurity of broiler flocks and thus prevent contamination of poultry with *Salmonella* and *Campylobacter* spp. can be difficult to implement and are also costly (Gibbens *et al*., 2001). Therefore, other approaches are required for use in combination with on-farm methods and these include treatments during processing that reduce the microbial burden on poultry carcasses.

Many studies have shown that the muscle tissue of a healthy animal is essentially sterile at the time of slaughter (Gill, 1979), with microbial contamination only present on the external surfaces. Until recently, a common technique that attempted to reduce this contamination was super-chlorination of cold rinse-water at the inside/outside wash stage. However, stringent regulations that limit the use of chlorination came into force in Europe in December 2003 (European Commission, 1998a). This has been a major factor in prompting the industry to investigate alternative methods of reducing the microbial load on poultry carcasses. Furthermore, concern regarding the use of chemicals has led to increasing interest in non-chemical, physical methods.

Many of these methods are heat-based. The rationale that explains why short-time heat treatments can kill bacteria, but need not cook the meat has been described by Morgan *et al*. (1996a). Heat kills bacteria mainly by inactivating

the most sensitive, vital enzymes. Typically, the heat of activation of these enzymes is 8.38 to 50.28 kJ g^{-1} mol^{-1}. The heat of activation for irreversible cooking of muscle is 209.5 to 419 kJ g^{-1} mol^{-1}, substantially higher. Only microgram amounts of enzyme need to be inactivated, compared to the grams of muscle denatured during cooking. 'For a square centimetre of surface contaminated with 100 bacteria, 15 million times as much heat is needed to cook the surface to a depth equal to the length of a bacterium, compared to the heat needed to kill all the bacteria' (Morgan *et al*., 1996a). Since bacteria are present only on the surface of the meat, even assuming that heating rates are the same for both bacteria and muscle means that, in theory, the bacteria should die before the meat is cooked. In fact, the meat will take longer, because it requires conductive heat transfer through the muscle.

17.2 Steam

Steam at 100 ºC has a substantially higher heat capacity than the same amount of water at that temperature. If steam is allowed to condense onto the surface of meat, it will rapidly raise the surface temperature. One very attractive feature of condensing steam is its ability to penetrate cavities and condense on any cold surface.

Water vapour molecules are much smaller than bacteria, for example 2×10^{-4} μm in diameter, compared with 0.7×4 μm for *Salmonella* cells (Morgan *et al*., 1996b). Therefore, steam is capable of reaching any bacteria that occur in cavities. Although the velocity of steam is reduced by cavities of diameter less than the mean free path of the gas density, this does not restrict steam in reaching bacteria. In 140 ºC saturated steam, the mean free path of the steam molecule is 0.4 μm, half the diameter of the smallest cavity capable of containing a *Salmonella* cell.

To prevent cooking, the steam must condense on the surface rapidly, and re-evaporate equally rapidly. Gases move by either flow or diffusion. Flow is rapid, motivated by a pressure gradient. Diffusion is much slower and motivated by a concentration gradient of the gas through other gases. During steam treatment, air, and any other non-condensable gas present, is concentrated by the inrush of condensing steam that forms a layer around the product surface. This prevents steam flow, slowing condensation as the steam diffuses through the layer. Non-condensable gases can come from three sources: gases around the carcass, when enclosed in a chamber; gases entering with the treatment steam and gases that have been desorbed by heat from the carcass or other surfaces.

The temperature at which water boils is a function of pressure. At atmospheric pressure, steam will be created initially at 100 ºC. At pressures below atmospheric (sub-atmospheric), the generation temperature will be lower than 100 ºC, while at pressures higher than atmospheric, it will be above 100 ºC. Generation at temperatures other than 100 ºC does not substantially reduce the heat capacity of the steam. Treatment temperatures below 100 ºC are less likely

to cause damage to the surface of the carcass, but will require longer treatment times than treatments at 100 ºC and above. One key disadvantage of both low- and high-pressure treatments is that they are batch systems.

17.2.1 Sub-atmospheric steam

Utilising the condensation of steam at sub-atmospheric pressure has been shown to be an effective method of decontaminating chicken drumsticks (Klose and Bayne, 1970). Reductions in TVCs of up to 5.6 log cfu per ml were achieved for drumsticks treated at 76 ºC for periods of up to 16 min, and the shelf-life at 3 ºC increased from 6 to 19 days. The work was later extended to surface pasteurisation of whole poultry carcasses (Klose *et al.*, 1971). Reductions of 3–3.7 log cfu per ml were achieved, using steam at temperatures not exceeding 75 ºC for four min. The orientation of the poultry carcass within the chamber and the method of introducing the steam were found to have significant effects on microbial counts. Toughening of the meat was observed, when steam treatment was applied to a chicken carcass within two hours of slaughter, but not if the steam was applied 22 hours after slaughter. This was possibly due to hot-shortening of the muscles.

Similar reductions in microbial counts were reported by Evans (1999). Steam at 75 or 85 ºC for 40 sec was required to reduce levels of *S.* Enteritidis inoculated onto chicken portions by 3–4 log units. However, some degree of cooking was apparent for all the meats investigated. On chicken, the outer layer of muscle was slightly cooked, although the skin was barely affected.

17.2.2 Atmospheric steam

The most successful steam process yet, in terms of industrial application, has been that developed in the USA by FMC Frigoscandia, the Steam Pasteurisation System (SPS) for red meat, using atmospheric steam. Studies on this commercially-available system for treating red-meat carcasses have been conducted and published by Kansas State University (Nutsch *et al*., 1997; Phebus *et al*., 1997). Significant reductions, of the order of 3.5 log units for specific bacteria, have been reported. The full commercial system (SPS 400 Steam Pasteurisation System) consists of a three-stage cabinet. Washed carcasses pass through an air-drying stage to remove residual water from the carcass, before an enclosed steam-treatment stage that is followed by spray-cooling.

A modified version of this system has been evaluated in a UK red-meat abattoir, and Whyte *et al*. (2003) carried out a series of trials on poultry carcasses. Conditions similar to those used to treat beef carcasses commercially in the USA (90 ºC for 12 sec) were found to produce statistically insignificant reductions in aerobic plate counts (APCs), Enterobacteriaceae and thermophilic campylobacters. Increasing the treatment time to 24 sec decreased counts by 0.75, 0.69 and 1.3 log cfu per g, respectively. However, visible damage to the outer skin tissue was found.

The effects of various steam treatments on the appearance, shelf-life and microbiological quality of chicken portions have been investigated at the University of Bristol (James *et al*., 2000). Application of steam at atmospheric pressure (100 ºC for 10 sec) on naturally-contaminated chicken breast portions resulted in a 1.65 log cfu per cm^2 reduction in TVC. However, in comparison with untreated controls, this treatment did not extend the shelf-life. Steam treatment for up to 10 sec on chicken portions inoculated with a nalidixic acid-resistant strain of *Escherichia coli* serotype O80 resulted in a maximum reduction of 1.90 log cfu per cm^2. Overall, results indicated that significant reductions in microbial counts could be achieved for chicken meat using steam. However, the reductions were less than would be expected from the time-temperature cycles used. Further studies (Corry *et al*., 2003) have continued, as part of a project funded by the UK Food Standards Agency, to devise practical methods that will reduce numbers of campylobacters and salmonellas on raw poultry carcasses and portions, without producing unacceptable changes in appearance or texture. Investigations have been carried out using a mixture of *C. jejuni* and *E. coli* K12 (a surrogate for *Salmonella)*, inoculated onto the breast skin of carcasses. Atmospheric steam-treatments reduced numbers of *C. jejuni* by about 1.5 log units in 10 sec, 2.5 log units in 12 sec and 3.5 log units in 20 sec. However, they also caused the skin to shrink and change colour. The optimum treatment for maximum effect on *C. jejuni*, least skin shrinkage and change of colour was concluded to be 12 sec. This work has shown that survival of the test strains of *C. jejuni* and *E. coli* K12 is similar, although campylobacters are usually considered to be more sensitive to heating and drying than either salmonellas or *E. coli.* Comparison of the heat-resistance of the test strain of *C. jejuni* with other strains of *C. jejuni* and *C. coli* indicated that the test strain was of average resistance.

Most researchers have aimed to develop processes that produce a substantial reduction in microbial numbers, but do not result in substantial cooking of the product. Avens *et al*. (2002) determined the treatment conditions necessary for a total thermal destruction of microbes (end count of < 10 aerobic microbes per cm^2), irrespective of damage. For atmospheric steam (96–98 ºC), a 3 min treatment was required to reduce natural contamination of retail carcasses from 10^4 cfu per cm^2 to <6. This treatment substantially cooked all the samples.

17.2.3 High pressure steam

Morgan *et al*. (1996a,b) have developed a device that permits surface treatment of meat at very high temperatures, without cooking. This system utilises very rapid cycles (for milliseconds) of heating and cooling, using steam under pressure and vacuum-cooling. Meat samples are placed in a rotating chamber. As it rotates, the meat is exposed to three other chambers that provide, respectively, a vacuum, steam and final vacuum. This procedure allows temperatures of up to 145 ºC to be used. Experimental trials demonstrated that treatment at 145 ºC for 25 millisec produced a 4-log reduction in *L. innocua*, added artificially to raw chicken meat.

The concept has been further developed by Kozempel *et al*. (2000, 2001, 2003a,b) as the vacuum/steam/vacuum (VSV) process. The initial prototype system, developed to treat whole carcasses (Kozempel *et al*., 2000), was found to have little significant effect on TVCs, when assessed using a whole-carcass rinse. Therefore, it was concluded that the abdominal cavity was not receiving a sufficient treatment. Consequently, the pilot system was modified to pass steam directly into the cavity (Kozempel *et al*., 2001). With this modification, the optimum process conditions (initial vacuum 0.1 sec, final vacuum 0.5 sec, vacuum absolute pressure of 4.1–7.1 kPa, steam time 0.1 sec, steam temperature 138 ºC) were shown to reduce counts of *L. innocua* by 0.7–0.8 log cfu per ml. The pilot system has been scaled up to a mobile unit (Kozempel *et al*., 2003a). Field studies of this system achieved a 1.4-log reduction for *E. coli* and a 1.2-log reduction for *Campylobacter* on naturally-contaminated carcasses; however, there was extensive mechanical damage caused by the introduction of steam into the cavity (similar problems were encountered in unpublished studies on a similar system at the University of Bristol). In further trials, the mechanical damage was eliminated and a 1.1–1.5-log reduction was obtained for *E. coli* K-12 (added artificially), with a total process time of 1.1 sec, although this period did not include the time taken to introduce and withdraw the product.

17.3 Hot water

Washing with water, either in the form of a spray or via complete immersion, are common decontamination treatments. The temperature of the water at the surface of the treated product and the method of applying the water are the two most important factors in removing microbes from carcasses. Spray-washing with cold water has been studied by a number of groups (Kotula *et al*. 1967; May, 1974; Mulder and Veerkamp, 1974; Notermans *et al*. 1980; Abu-Ruwaida *et al*. 1994). Washing poultry carcasses in this way has been shown to be effective in removing physical contaminants, such as soil, feathers and other debris, but its effect on microbial numbers is slight. Physically removing bacteria by using cold-water sprays is only partially effective, since attached/entrapped bacteria have been shown to be particularly difficult to dislodge (Firstenberg-Eden, 1981; Lillard, 1989; Abu-Ruwaida *et al*., 1994). Studies by a number of workers have shown that immersion of carcasses causes swelling of skin and tissues, and absorption of bacteria into deep tissues, making them even more difficult to remove by washing (Notermans and Kampelmacher, 1974; McMeekin and Thomas, 1978; Thomas and McMeekin, 1982; Lillard, 1989). Raising the temperature of the water increases the reduction in microbial counts. However, there are practical problems in using hot water, for instance, a spray-jet rapidly loses heat by evaporation. Studies have shown that the maximum impact temperature on the carcass from a spray placed 30 cm away and supplied with water at 90 ºC is approximately 63 ºC (Bailey, 1971).

While it is generally accepted that washing is an effective method of removing visible contamination from meat carcasses, there are persistent fears that it may merely redistribute microbes over the carcass. Trials on sheep carcasses have shown that washing led to microbial contamination of the dorsal area, which was not contaminated beforehand (Ellerbroek *et al*., 1993). Washing of beef carcasses has also been shown in one study to bring about a 'posterior to anterior redistribution' (Bell, 1997). However, another study on beef carcasses (Charlebois *et al*., 1991) found little change in the distribution after spray-washing.

Decontaminating poultry meat by using either hot-water sprays or immersion methods has been studied by a number of researchers, with some degree of success (Dawson *et al*., 1963; Pickett and Miller, 1966; Avens and Miller, 1972; Cox *et al*., 1974a,b; Teotia and Miller, 1972; Thomson *et al*., 1974, 1979; Morrison and Fleet, 1985; de Ledesma *et al*., 1996; Berrang *et al*., 2000; Li *et al*., 2002). As would be expected, it appears from the literature that treatments are more effective at higher temperatures and with longer immersion or exposure times. Up to 2-log reductions have been obtained in some of these studies (Pickett and Miller, 1966; Cox *et al*., 1974a; Morrison and Fleet, 1985). Although substantial reductions may be achieved with water temperatures exceeding 60–65 ºC, such treatments can result in a partially cooked product, with browning of the flesh and tightening of the skin of the carcass.

While data are available on the death kinetics of pathogenic bacteria, there is little information on the relationship between surface temperature and the appearance and colour of poultry tissues. The data of Morgan *et al*. (1996a,b) show that it is possible to expose small pieces of chicken breast (10 mm × 10 mm, 50 mm long) to steam at 100 ºC for 1000 millisec, without cooking. These workers also provide a clear table of cooking times at higher temperatures. Data for lower temperatures are less clear. Morrison and Fleet (1985) reported that carcasses immersed in water at 60 ºC for 10 min were acceptable. Dawson *et al*. (1963) found that water at temperatures higher than 60 ºC caused partial cooking. Cox *et al.* (1974a) showed that immersion of carcasses at 71.7 ºC greatly impaired their appearance.

Göksoy *et al*. (2001) carried out investigations to determine the maximum times that chicken-breast samples could be immersed in water at temperatures between 50 and 100 ºC, before the appearance of the samples changed irreversibly. The data produced show that these times range from 120 sec at 50 ºC to 1 sec at 100 ºC. All the samples in this study were packed in a polyethylene film. While it is probable that the rate of temperature rise at the surface of unwrapped samples would be higher, the exposure time before noticeable changes occur is likely to be lower than those reported. Most of the changes in appearance caused by the heat treatments were to the cut edges and exposed muscle of the samples. Similar observations were recorded by Morgan *et al*. (1996a). It would be expected that whole poultry carcasses could stand longer immersion times, since the intact skin would offer more resistance to heat penetration and that is the case in conventional carcass scalding. Temperature

analysis showed that, at the lower temperatures and longer treatment times (50 ºC for 480 sec and 60 ºC for 300 sec), the temperature measured below the skin almost reached that of the surrounding water. However, the short immersion times at higher temperatures were not sufficient to allow heat to penetrate through the skin. This is not unexpected, since chicken skin has a relatively low thermal conductivity, $0.357\,W\,m^{-1}\,K^{-1}$ (Morley, 1972) and similar results were reported by de Ledesma *et al*. (1996). The latter found that the subcutaneous temperature of a chicken wing, with an initial temperature of 4.2 ºC, reached a maximum of 11 ºC after immersing the wing in water at 95 ºC for 5 sec. Tests on *E. coli* serotype O80, added artificially, and utilising treatments that had no effect on visual quality were found to be ineffective in reducing microbial counts.

Subsequent work by Purnell *et al*. (2004) developed and evaluated an experimental in-line processing unit for poultry carcasses, using hot-water immersion. Treatment at 75 ºC for 30 sec significantly reduced APCs and counts of Enterobacteriaceae and campylobacters, but the skin tended to tear during trussing. However, treating carcasses at 70 ºC for 40 sec, followed by a 12–15 ºC spray-chill treatment for 13 sec, did not detrimentally affect the skin and microbial counts remained significantly lower than the controls for eight days under typical chill-storage conditions. After heat treatment at 70 ºC for 40 sec, the proportion of carcasses graded as unacceptable, due to epidermal damage during the treatment and skin tears during the trussing process, constituted 5% and 9%, respectively, for two separate batches. Clearly, this proportion of unacceptable carcasses would have serious commercial implications, and could limit the application of the method. A continuation of this work, as part of a project funded by the UK Food Standards Agency (as yet unpublished), has shown that reductions in added *E. coli* and *C. jejuni* of the order of 1.3 log cfu per cm^2 can be achieved, using the treatment temperatures and times identified by Purnell *et al*. (2004) as acceptable.

17.4 Dry heat

While many techniques have involved the application of wet heat in order to decontaminate meat, few have utilised dry heat. The use of rapid desiccation by means of dry heat from a forced-air heater has been studied by Cutter *et al.* (1997). This laboratory study on beef carcasses examined combinations of desiccation at 400 ºC or 300 ºC, before inoculation of carcasses with bovine faeces, with subsequent washing in water at 35 ºC. Combined treatments were found to be more effective than washing alone. The authors postulated that the desiccation process affected the meat surface in such a way that the degree of bacterial attachment was reduced.

A limited study (Corry *et al*., 2003) at the University of Bristol used an experimental, pilot-scale system to evaluate controlled, hot-air heating of food surfaces (James and James, 2003). Results showed large reductions in counts of

added *C. jejuni* and *E. coli* from chicken skin subjected to 15 min treatments with high velocity (*c.* 15 m per sec) warm air at 10, 40, 50 and 60 ºC. Reductions of 1–2 log units at 20 and 40 ºC indicated that there was some form of non-thermal drying effect. However, these data have not been fully validated.

17.5 UV light

The potential of ultra-violet (UV) radiation in retarding growth or even killing microorganisms has been known since the latter half of the nineteenth century (Haines and Smith, 1933). Commercial use of UV to extend the storage-life of chilled red meat has been reported from the 1930s onwards (Haines and Smith, 1933; Ewell, 1943). Many reports show that exposure to UV can reduce surface contamination of meat by 2–3 log cfu per cm^2 and it would appear to have no deleterious effects on the appearance of the meat. Under high intensity UV, exposure times would be <10 sec. UV appears suitable for on-line decontamination of meat, either as carcasses, primals or retail cuts. To achieve an even exposure at all points on the surface of the meat appears to be the main technical problem. The use of robotics and automation to orientate the meat with the source or move the source(s) over the surface to prevent 'shadowing' needs to be examined.

The lethal effect of UV varies with the intensity of the radiation and the time of exposure. Temperature, pH, relative humidity and degree of microbial contamination influence the lethality of UV radiation (Banwart, 1989). UV has low penetrating power. Areas of shadow, dust in the air and the clumping of microbial cells all have a protective effect. However, the production of ozone by certain wavelengths of UV gives an added germicidal effect (Banwart, 1989; Kaess and Weidemann, 1973). Levels of ozone should be limited to < 0.3 ppm, as high concentrations can cause rancidity (Anon., 1970). UV may cause an acceleration in lipid oxidation and the formation of brown metmyoglobin pigment in lean muscle. This is more pronounced in pork and poultry, and therefore it has been suggested that UV treatment should be restricted to beef, veal, mutton and lamb (Anon., 1970).

The most effective, bactericidal UV wavelengths are between about 240 and 280 nm (Sykes, 1965); at these wavelengths, UV is readily absorbed. In practice, a radiation wavelength of 253.7 nm is most commonly used, as 90% of the radiation emitted from UV lamps consists of this wavelength (Sykes, 1965).

Sykes (1965) reported that Gram-negative bacteria are the most sensitive to UV, then Gram-positive cocci, with spores of bacteria and moulds being the most resistant. Banwart (1989) states that, generally, UV is equally effective against yeasts, Gram-positive and Gram-negative bacteria. Chang *et al.* (1985) found UV inactivation of *E. coli* to be comparable to that for *Staph. aureus* and *S.* Typhi, all requiring approximately 7 mW sec per cm^2 for a 3-log reduction, while *Strep.* (*Enterococcus*) *faecalis* required 1.4 times this dose for a comparable inactivation. Butler *et al.* (1987) found *E. coli* to be more resistant than

either *Y. enterocolitica* or *C. jejuni*, dose requirements for a 3-log reduction being 5.0, 2.7 and 1.8 mW sec per cm^2, respectively.

Experiments conducted by Yndentad *et al*. (1972) and Wallner-Pendleton *et al*. (1994) showed that doses of 10,000 μWsec per cm^2 for 12.8 sec and 82,560 μW sec per cm^2 for one min respectively did not extend the shelf-life of poultry meat. Experiments carried out with *Salmonella*-inoculated chicken samples have shown that doses up to 86,400 μW sec per cm^2 (254 nm wavelength) result in less than a 1-log unit reduction in the numbers of added *S.* Typhimurium on chicken skin and halves (Wallner-Pendleton *et al*., 1994; Sumner *et al*., 1996). However, Purnell and James (2000) found that reductions of up to 1.9 log cfu per cm^2 in APCs could be achieved for skin-on chicken breasts exposed to a 3.4–3.7 mW per cm^2 treatment for 10 sec.

In general, UV radiation alone is not a suitable decontamination method for poultry carcasses, because the low penetration of UV restricts its ability to destroy bacteria located in crevices and follicles. Other surface characteristics of chicken skin may also reduce the effectiveness and uniformity of UV decontamination. However, there is conflicting evidence in relation to the potential of UV for treating chicken portions.

17.6 Microwaves

A number of reports imply or claim that microwave exposure has an athermal antimicrobial effect on bacteria, although many other studies have concluded that any effect of microwaves is solely due to heat generation. In their review of the subject, Fung and Cunningham (1980) came to no clear conclusion. However, Heddleson and Doores (1994) concluded that microwaves have solely a thermal role to play in destroying bacteria.

A number of researchers have studied the use of microwave energy to reduce microbial counts from poultry meat (Teotia and Miller 1975; Cunningham and Albright, 1977; Cunningham, 1978; 1980; Göksoy *et al*., 1999; 2000) and vacuum-packed beef (Fung and Kastner, 1982; Kenney *et al.*, 1995; Paterson *et al.*, 1995). Teotia and Miller (1975) found that microwave exposure for 600 and 120 sec was required to destroy *S.* Senftenberg on broiler carcasses and turkey drumsticks respectively. However, this treatment resulted in partially-cooked meat. The frequency and power output of the oven that was used were not reported. Cunningham and Albright (1977) applied microwaves at 2450 MHz (the frequency used by domestic microwave ovens) to chicken portions and skin for 10, 20 and 30 sec. They reported that, in most cases, exposure for 30 sec reduced TVCs from chicken portions by approximately 2 log units, while causing some moisture loss and increasing the meat temperature 'considerably'. It was also observed that skin samples exposed to microwaves for 30 sec had TVCs of less than 5×10^3 per cm^2 after storage at 4 ºC for seven days, whereas counts on untreated samples stored under the same conditions reached 10^5 after only five days. In further studies

(Cunningham, 1980), exposure of chicken portions for 40 sec, using a frequency of 915 MHz, also reduced counts by about 2 log units, while coliform counts were reduced by 50% after treatment for 20 sec. Experiments involving the application of 2450 MHz microwaves to skin samples produced similar results. Meat treated for less than 20 sec showed 'no drastic changes in appearance or physical characteristics'. Such treatment reduced numbers by about 1 log unit and extended the shelf-life. Cunningham (1980) concluded that minimal microwave exposure might be used to extend the shelf-life of poultry or other meat products. He suggested that consumers could extend the storage-life of fresh meats bought in supermarkets by giving them microwave treatment for 15–20 sec before placing them in a refrigerator.

Fung and Kastner (1982), using microwaves of unstated wavelength and an oven of unstated power, reported that vacuum-packed beef treated for 5–20 sec had lower microbial counts after storage and display, and better odour scores, than untreated controls. Paterson *et al*. (1995) submerged vacuum-packed beef steaks in warm (40 ºC) water to reduce edge heating during processing in a domestic microwave oven (2450 MHz, 700 W at maximum power). Processes that achieved a surface temperature of 55 ºC resulted in an acceptable product and a 2-log cfu per cm^2 reduction in microbial numbers. In contrast to these studies, Kenney *et al*. (1995) reported that microwave treatment of vacuum-packed joints of beef (using 2450 MHz, output wattage of 112.9 per Wh) resulted in very variable surface temperatures, 5–80 ºC on sample edges and 5–50 ºC at the centre, and no significant effect on microbial quality.

While the number of papers on the use of microwave energy for decontaminating meat suggest that the technique is promising, work at the University of Bristol (Göksoy *et al*., 2000) refutes the claims made. This work used domestic microwave ovens and showed microwave treatment to be too uneven and unreproducible to heat the meat surface without cooking. Fresh skinless chicken breasts were inoculated with *E. coli* K12 and *C. jejuni* (5–6 log cfu per cm^2) and exposed to microwaves (2450 MHz) in an experimental microwave oven operated at full power (IEC 1138.8 W) for 10, 20 or 30 sec. All three exposures had only a minimal effect on bacterial numbers and, in some cases, counts were higher after treatment, irrespective of treatment time. Exposure to microwaves for 20 and 30 sec had some effect on meat appearance (signs of partial cooking were observed). Further trials were carried out using uninoculated skin-on breasts to determine whether exposure for 30 sec had any effect on subsequent microbial growth and hence shelf-life. There was found to be no difference between the shelf-life of treated and untreated samples stored at 3 ± 1 ºC. Overall, the results indicated that short-time exposure of microorganisms on chicken meat to microwaves has no significant effect on microbial numbers or subsequent growth. The results confirm those of a previous study (Göksoy *et al*., 1999) and suggest that microwave heating is not sufficiently controllable for decontamination purposes.

17.7 Ultrasound

The physical and biological effects of high-frequency sound waves and their lethal effects on bacteria were described in the 1920s (Sykes, 1965). Ultrasound is most effective against microorganisms in liquid suspension, the lethal action being attributed to physical destruction of the cells through 'cavitation', which produces extreme fluctuations of pressure and temperature within the liquid. This causes physical damage to the microorganisms. The efficiency of treatment is related more to the intensity of the wave than to frequency. Cavitation is more difficult to induce and less intense, as frequency is increased (Sykes, 1965). In general, coccoid organisms and Gram-positive bacteria are more difficult to kill than Gram-negative and rod-shaped forms; also, anaerobic organisms are more sensitive to ultrasound than aerobes (Sykes, 1965; Ahmed and Russell, 1975). In addition, it has been reported that ultrasound is capable of inactivating toxins as well as microorganisms (Banwart, 1989).

Few studies have been made on the use of ultrasound in food systems (Sams and Feria, 1991). Most antimicrobial applications have been for liquid foods, such as milk. Since ultrasound is most effective in liquid systems, it appears that immersion chilling of poultry is a process where ultrasound could be applied, both in treating the processing water and decontaminating the carcasses. Sams and Feria (1991) found ultrasound (47000 Hz; 200 W output), and ultrasound in combination with lactic acid and/or heat, to be ineffective in reducing microbial counts from the surface of broiler drumsticks. The lack of antimicrobial effect was attributed to the irregular skin surface providing some degree of physical protection against cavitation. In contrast, studies by Lillard (1993, 1994) showed ultrasound to be effective in reducing numbers of *Salmonella* attached to broiler skin. Also, *Salmonella* in peptone was reduced by 1–1.5 log during sonification at 20 kHz for 30 min, by < 1 log using chlorine alone, and by 2.5–4 log during sonification in a 0.5 ppm chlorine solution (Lillard, 1994). A number of possible explanations were offered for the difference between the results of the latter studies and those of Sams and Feria (1991). These included, the use of skin pieces v. whole drumsticks, peptone v. deionised water and the synergistic effect of combining ultrasound with chlorine.

17.8 Drying during chilling

There is some evidence that drying the surface of the carcass, which occurs sometimes during air-chilling, inactivates any campylobacters present (Doyle and Roman, 1982; Oosterom *et al*., 1983; Bolder and van der Hulst, 1987; Allen *et al*., 2000a,b), although this does not seem to be a reliable effect. The microbiological aspects of meat chilling were reviewed by Shaw *et al.* (1986) and it was concluded that 'a systematic study including a range of chill temperatures, relative humidities and air speeds has yet to be performed and it is therefore still difficult to predict the effect of different chilling systems on overall bacterial

numbers on carcass surfaces'. This continues to be the case with respect to the chilling of all kinds of meat, including poultry. Microbial aspects of poultry refrigeration are further discussed in Chapter 14.

17.9 Other novel techniques

A whole range of more novel techniques, such as the use of visible light (Mertens and Knorr 1992), have been suggested for treating poultry meat, and in some cases shown to be viable alternatives. Most of these methods depend on heat to destroy the bacteria present, although a number of non-thermal treatments have been proposed (Mertins and Knorr, 1992). Many of the alternative physical treatments rely on the effect of radiant energy on surface-located bacteria (Table 17.1). These methods include the use of visible light and lasers. Others rely on the effect of electromagnetic fields and include electrical stimulation (ES), high-voltage, pulsed electric field (PEF) and oscillating magnetic-field pulses (OMF). In addition, use of high pressure and air ions have been investigated.

Very brief, high-intensity pulses of visible light produce a > 1 log reduction in microbial numbers. However, few data are currently available on the process. Attaining very high surface temperatures for a very short period by means of lasers might also have much to offer in the future.

ES appears to produce only a small reduction in microbial levels in laboratory studies on model foods, but reductions of up to 6 log cfu per cm^2 have been reported, following the application of high voltage PEF (Zhang *et al*., 1994). The technique is currently in its infancy and will require considerable development before it can be applied even to small pieces of meat. Similarly, the application of OMF appears to be an effective means of destroying bacteria, especially in liquid foods, but is unlikely to have any application for decontaminating meat in the near future (Mertens and Knorr, 1992).

Laboratory trials have shown that very high pressure processing is an effective method of extending the chilled storage life of highly contaminated

Table 17.1 Mode of antimicrobial action for different physical decontamination treatments

Method	Mode of action on microbial cells
Steam	Thermal effect
Hot water	Thermal effect and physical removal
Dry heat	Thermal and desiccation effects
Ultraviolet light	UV effect
Microwave	Thermal effect
Ultrasound	Rupture of cell membrane
Pulsed light	UV (or thermal) effect
Ultra high pressure	Denaturing of protein
Pulsed electric fields	Rupture of cell membrane

minced meat (Carlez *et al*., 1994). It also reduces significantly the risk of survival for pathogenic microorganisms. However, the cost of high-pressure equipment that could process substantial quantities of meat appears to limit its commercial uptake.

IR heating has been used successfully to reduce microbial counts on the surface of pig carcasses after scalding and dehairing operations have been completed (Snijders and Gerats, 1977). Carcasses were exposed for 90 s, raising the skin temperature to 150 ºC. Surface discolouration and drying occurred, but the appearance later reverted to normal.

17.10 Selecting the right technique

The shape of a poultry carcass is not ideal for decontamination. Most decontamination treatments rely on physical contact and uniform coverage of the carcass surface. This is difficult, since the surface of a poultry carcass is irregular and there is an abdominal cavity that is reached through a relatively small opening. Also, the outer surface of a carcass has many crevices and folds. These areas are very difficult to treat and tend to provide protection for attached bacteria. They slow down the penetration of both liquid and gaseous treatments and cause shadowing problems for radiation methods, such as UV light. As well as protecting bacteria, these areas often acquire physical debris and do not drain well. Accumulation of water or chemical solutions lying in these areas can have a detrimental effect on the visual quality of the carcass and cause difficulties in controlling the treatment contact time.

There is much evidence that the time after slaughter at which carcasses are treated greatly affects the efficacy of a decontamination process. The longer bacteria reside on the carcass surface, the more difficult removal becomes, because of their ability to attach to tissue. However, organisms differ in their ability to attach to different surfaces and in the time they require to become fully attached. The formation of biofilms may increase microbial resistance to destructive treatments.

There is rarely any distinction in the literature between decontamination ‘methods’ (i.e. the method of applying a treatment) and decontamination ‘treatments’. This often clouds the practical issues of decontamination. Too much emphasis tends to be placed on the treatment rather than the method of application. Decontamination is not a matter of simply dipping or spraying the product with chemicals or water, or giving it a brief flash of light or exposure to steam. For example, many factors affect the efficiency of aqueous spray systems. In automated spray cabinets, the position and number of the sprays, the shape of each spray and spray pressure, all have a significant effect on the treatment, irrespective of the nature of the solution being pumped through the sprays. Some studies, however, have shown that a deluge method of application, where the carcass is passed under a waterfall, offers a more effective method of coverage (Davey and Smith, 1989). Many studies have indicated that the method of decontamination is often more important than the treatment itself.

Heat treatments, with or without chemicals, are very reliant on the method of application. To prevent cooking of the product, such treatments have to provide a uniform heating of all surfaces for a short period. This is not particularly difficult to achieve on a laboratory scale by spraying or dipping small samples, using hot water, for example. Similarly, laboratory studies using steam have shown that, if very high temperatures are applied for very short times, followed by rapid cooling of the surface, large reductions in microbial contamination can be achieved, without affecting the appearance of the meat. However, successfully applying such techniques to carcasses in a processing plant presents many engineering challenges.

17.11 Future trends

Poultry carcasses typically yield TVCs between 10^2 and 10^4 cfu per cm^2 (Corry *et al.*, 2003). Obviously, a 4-log reduction would almost guarantee a sterile carcass. To date, no adequate method of achieving this has been found, without affecting the sensory quality of the meat. Also, no treatment, as yet, can be relied upon to eliminate all pathogens, although smaller reductions, over and above those achieved in the basic process, would still be of value. In relation to *Campylobacter*, it has been predicted that a 2-log reduction would lead to a 30-fold decrease in human campylobacteriosis (Rosenquist *et al*., 2003).

With the increase in commercial interest and use of decontamination treatments for red meats (particularly in the USA), more and more studies are being carried out in operational abattoirs, unlike much of the earlier work that was often confined to a bench scale. In the USA, commercial red-meat abattoirs are utilising a wide range of decontamination treatments, often sequentially. Steam is being applied commercially to red meat in the USA and undergoing industrial trials for poultry in the UK. Despite the success and commercial realisation of practical steam-pasteurisation systems, there are still gaps in our understanding of these systems. To realise the full potential of surface pasteurisation by steam, it is necessary to understand the relationship between heating and cooling cycles on the one hand and any quality changes in the foods of interest on the other. The treatments that maximise microbial reduction, without significant quality changes, need to be identified, along with the development of engineering expertise to produce the required conditions consistently over the surface of the food being treated, at industrial rates of throughput. While effective commercial systems are now available, there is much evidence that these systems still require further development to achieve full efficiency.

The use of decontamination treatments by the poultry industry in the EU remains restricted by legislation that defines what can or cannot be permitted. Meanwhile, the adoption of a ‘zero tolerance’ requirement in the USA for *E. coli* has effectively forced the American red-meat industry to use antimicrobial systems. Thus, the introduction of efficient antimicrobial systems in other countries may be required, simply to maintain current export markets. In

response to American policy, Australian and New Zealand meat plants are also investigating and developing antimicrobial systems.

It is debatable whether consumers would be willing to pay more for safer food. They believe logically that food is already safe. However, processes that eliminate pathogens should also produce a substantial reduction in the numbers of spoilage organisms and hence an extension of product storage-life. This would help the economics of food production, allowing longer production runs, delivery to more distant markets, and reduced waste. In fact, the introduction of surface pasteurisation does not directly improve profitability by cost savings or increased throughput. Consequently, despite the obvious advantages to the industry as a whole and the consumer, such systems will only be introduced if they are cheap, reliable and have low running costs. Surface pasteurisation with atmospheric steam has the potential to meet these requirements.

In conclusion, any decontamination system for meat that is adopted in the EU will depend on a perceived need by government and food retailers, and may require changes to current legislation. The EU Scientific Committee on Veterinary Measures Relating to Public Health has published an opinion regarding the decontamination of poultry carcasses (European Commission, 1998b). The Committee recommended that:

1. Antimicrobial treatment should only be used as part of an overall strategy for pathogen control throughout the whole production chain.
2. Before any decontamination compound or decontamination technique is authorised for use it should be fully assessed.
3. The person or company proposing such a decontamination compound or decontamination technique must demonstrate that all aspects have been covered.
4. The person or organisation using a decontamination compound or decontamination technique must demonstrate that effective control of parameters critical for efficacy and safe use are in place, and that good practice and appropriate HACCP plans are implemented.

Future developments in on-line decontamination of poultry carcasses will need to address these recommendations.

17.12 Sources of further information and advice

An extensive review of decontamination methods for meat has been published by James and James (1997) and is now regularly updated at the University of Bristol. Good general reviews of contamination and decontamination issues have been published in recent years by Corry and Mead (1996), Bolder (1997), Dorsa (1997), Sofos *et al*. (1999) and Bjerklie (2000). An overview of contamination and decontamination issues involving poultry meat has been produced by Smulders (1999). Useful books include those edited by Gould (1995), Smulders (1987) and Ellerbroek (1999).

Reviews of specific subjects include Jeyamkondan *et al*. (1999) on pulsed electric-field processing, and Mertens and Knorr (1992) and Palmieri *et al*. (1999) on non-thermal preservation methods (such as pulsed electric fields, pulsed light and oscillating magnetic fields).

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18

Microbial treatments to reduce pathogens in poultry meat

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18.1 Introduction

This chapter will focus on treatments to reduce the incidence of pathogens in poultry and specifically those involving the actions of microorganisms. Within the definition of 'therapeutic', we will concentrate mainly on microbial treatments that inhibit *Campylobacter* and *Salmonella*, as these bacteria represent the biggest challenge to the poultry industry in terms of reducing human foodborne disease. We will also include therapeutic treatments of poultry and other animals that may lead to the prevention of avian diseases in the future. The two microbial treatments to be covered in detail are those involving the use of bacteriophage or bacteriocins. The history, essential characteristics, practical considerations and potential shortcomings of each will be described, along with specific examples of their application. It should be noted that these methods, under-researched until recently, have become fuelled by the increase in antibiotic resistance, coupled with a public desire to reduce the use of chemicals in the food we eat. These factors have led researchers to re-evaluate nature's ways of controlling bacteria. We will not include a detailed account of competitive exclusion (CE) strategies although, strictly speaking, these are microbial treatments, because they are discussed elsewhere in this volume. The inhibitory effect of CE, using mixed populations of largely undefined bacteria, is thought to be due to a combination of factors, including competition for colonisation sites, competition for nutrients, stimulation of immunity and production of toxic metabolites that include bacteriocins (Patterson and Burkholder, 2003). In this chapter, however, we will consider the use of defined bacteriocin-producing bacteria as microbial treatments in their own right.

Microbial treatments can be administered to the live birds or applied to poultry carcasses during processing to reduce the presence of pathogens on the final product. There are few reports of successful microbial treatments for live animals. Those involving bacteriophage are reviewed by Barrow (2001) and Payne and Jansen (2003). There are even fewer relating specifically to poultry, reviewed by Joerger (2003), including use of bacteriophage and bacteriocins.

18.2 Bacteriophage

Bacteriophage, often simply called phage, are defined as viruses that can infect, multiply and kill susceptible bacteria. Bacteriophage are ubiquitous in the environment and are found wherever suitable bacterial hosts are available to them. Estimates place the number of phage in the biosphere at 10^{31} phage particles, making them the most abundant biological entity on the planet (Hendrix *et al.*, 1999). Twelve families of phage are recognised, based principally on their morphological characteristics and nucleic acid content (Ackermann, 2001). The most frequently encountered bacteriophage are the double-stranded DNA, tailed phage, with icosahedral heads. Figure 18.1 shows an electron micrograph of a typical *Campylobacter* bacteriophage, belonging to the Family Myoviridae, that have genome sizes of approximately 140 kb (bar represents 0.1 μm). Less frequently encountered bacteriophage may have single-stranded DNA or single-stranded RNA and a variety of morphological forms, for example the filamentous phage (Adams, 1959).

18.2.1 Bacteriophage therapy

The idea of harnessing the potential of bacterial viruses to treat bacterial infection (phage therapy) has been around for many years (D'Herelle, 1922), arising not long after the discovery of bacteriophage (Twort, 1915). Former

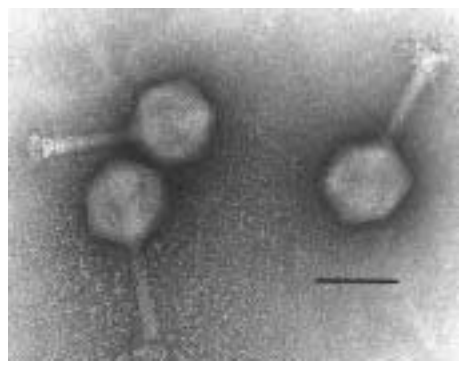

Fig. 18.1 Electron micrograph of a typical *Campylobacter* bacteriophage (Family Myoviridae). Bar represents 0.1 μm.

Warsaw Pact countries have exploited the use of bacteriophage for therapeutic, prophylactic and disinfection purposes for many years, reviewed by Alisky *et al.* (1998). However, it is only since the dramatic rise in multi-drug resistant bacteria that Western scientists have looked to phage therapy as an alternative to combat infection; see the recent reviews by Sulakvelidze and Morris (2001) and Summers (2001).

The success of phage therapy or the use of phage as specific agent of disinfection is very much dependent on the selection of the phage to be used. The phage must be lytic and demonstrate high virulence against the target bacteria. Lytic phages, such as the T-even phage of *Escherichia coli*, lyse bacterial cells as a consequence of their life cycle or fail to replicate. They always infect from outside, upon which they reprogramme the host cell to produce more phage particles and release these as a burst of phage through cellular lysis, after a relatively fixed interval. In contrast, lysogenic or temperate phages are generally unsuitable for phage therapy, as they can integrate their DNA into the host DNA and render the host bacterium immune to further infection through the production of a phage-encoded repressor. The repressor actively prevents the expression of the phage's own genes and those of any other related phage that may subsequently infect. Furthermore, lysogenic phage are prone to transduction, the phage-mediated transfer of genetic material from one bacterial host to another. This form of DNA transfer may include the dissemination of pathogenic traits amongst their hosts (Cheetham and Katz, 1995; Boyd and Brussow, 2002). For example, shiga-like toxin-producing strains of *E. coli*, that include O157:H7, carry the toxin-encoding genes on lamboid prophage that are integrated into the bacterial genomic DNAs (Scotland *et al.*, 1983; O'Brien *et al.*, 1984). As a result, the expression of the toxin or toxins is under regulatory control, as if they were phage late gene products, with the consequence that any factor that will commit the prophage to excise and initiate lysis will result in the increased expression of the toxin (Smith *et al.*, 1984). These factors include stress, such as antibiotic therapy or even pre-existing lambdoid phage populations, if suitable host bacteria are available (Gamage *et al.*, 2003).

Having selected the appropriate lytic phage, the approach confers several advantages as discussed by E. Kutter (http://www.evergreen.edu/phage/phagetherapy/phagetherapy/htm):

- Phage are both self-replicating and self-limiting, since they will multiply only as long as sensitive bacteria are present, and then are gradually eliminated from the environment.
- The specificity of the phage means there will be less damage to the normal microbial flora; this is particularly important in the gut. Bacterial imbalance or 'dysbiosis' caused by antimicrobial treatment can cause a number of problems that are difficult to treat, as the residual bacteria are often quite resistant to further antimicrobials.
- Phages can be selected to target surface receptors that are involved in pathogenesis, so that any residual population is attenuated in virulence.

- As phage are often already present in the environment, in equilibrium with the host bacterium, the likelihood of phage treatment associated side effects such as allergic response are remote, when compared to antimicrobial intervention strategies.
- Phages are fairly inexpensive to prepare, although consideration must be given to microbiological protection of production staff that may be exposed to pathogenic hosts (Joerger, 2003).

Adoption of phage therapy has been held back by a lack of consistent proof of efficacy. Previous failures may have been due to a general lack of understanding of the kinetics of phage replication, which is a density-dependent process to which mathematical models can be applied (Levin and Bull, 1996; Payne and Jansen, 2001; Bull *et al.*, 2002). Phage replication is critically dependent on the density of bacteria present. There is thought to be a distinct threshold above which phage numbers increase and below which they decrease, termed the 'phage proliferation threshold' (Wiggins and Alexander, 1985; Payne and Jansen, 2003). This threshold may need to be higher *in vivo* due to the potentially greater rate of phage loss (Payne and Jansen, 2002). The outcome of phage therapy also depends on various life-history parameters, including the inoculum size, inoculum timing, phage absorption rate and burst size (Levin and Bull, 1996; Payne and Jansen, 2001; Weld *et al.*, 2004). It is difficult to translate information gained from the homogeneous bacteria and phage in a well-mixed and controlled environment to the situation *in vivo*. The intestinal lumen is a complex environment, where various physical (for example, constant flow of material), physiological (for example, levels of oxygen), host defences and biochemical factors (such as pH) all influence the populations of colonising bacteria. In addition, the kinetics of phage absorption in the intestine may be quite different from those in laboratory media, due to the viscosity of the mucus layer (Weld *et al.*, 2004).

If phage are mixed with bacteria in ratios where phage greatly outnumber bacteria (high multiplicity of infection, MOI), bacteria may be 'lysed from without', largely due to destabilisation of bacterial membranes. This may lead to an initial drop in titre of both bacteria and phage, as free phage may adhere to the large amounts of bacterial-cell debris rather than healthy cells (Rabinovitch *et al.*, 2003). This strategy is known as 'passive inundation', where phage simply overwhelm the bacteria and do not replicate (Payne and Jansen, 2001). There have been several reports of successful phage treatments with high doses of phage, for example, *Vibrio vulnificus* was controlled in an iron-dextran-treated mouse model by administration of 10^8 plaque forming units (PFU) per ml (Cerveny *et al.*, 2002). Also using a mouse model, the application of high phage doses in the treatment of vancomycin-resistant *Enterococcus faecium* was found to be most effective (Biswas *et al.*, 2002). Another possible strategy, known as 'active proliferation' (Payne and Jansen, 2001), involves the provision of a low initial dose of phage that then replicate on proliferate target bacteria. The result is an eventual decline in numbers of bacteria, when phage numbers have

replicated sufficiently. This has the advantage that less starting material is required, but may allow time for resistance to be acquired by mutation. An example of this strategy was demonstrated by Soothill (1992), in which mice treated with 10^2 particles of an *Acinetobacter* phage were able to protect other mice against a virulent strain of *A. baumanii*. The phage was shown to have multiplied in the mice under challenge.

Factors that may affect phage efficacy *in vivo* include: host defences, proteolytic enzymes and physical factors, such as absorption to food particles, or non-host bacteria may reduce the titre significantly (Rabinovitch *et al.*, 2003). Even if the phage can replicate, they do not always affect the desired reductions in the enteric bacterial population. For example, phage active against *S.* Typhimurium could be shown to multiply in chicks challenged by the bacterium, but phage replication did not affect the numbers of *Salmonella* recoverable from the caeca (Berchieri *et al.*, 1991).

The selection of resistant bacteria has always been perceived as a potential obstacle to phage therapy (Barrow, 2001), and has been reported following experimental phage treatments (Smith and Huggins, 1982; Smith *et al.*, 1987a; Sklar and Joerger, 2001). However, phage resistance can be acquired at a price, such as a reduction in the colonisation potential or virulence of an organism. For example, phage-resistant types that survive treatment by bacteriophage against the fish pathogen *Pseudomonas plecoglossicida* were less virulent when used to reinfect susceptible fish (Park *et al.*, 2000). Selecting phage that target a virulence factor, such as the capsular antigen (K) of *E. coli*, has proved to be of particular value, since the number of resistant variants isolated following phage treatment can be low (Smith and Huggins, 1983; Smith *et al*., 1987a; Levin and Bull, 1996). Evidence against the dominance of phage-resistant populations can be gained from the examination of natural phage infections. We have recently conducted a longitudinal study of a broiler chicken house naturally infected with *Campylobacter* and phage over three successive rearing cycles (Connerton *et al.*, 2004). Occasional, phage-resistant *Campylobacter* strains could be isolated, but these did not dominate or outgrow the sensitive types; instead, they co-existed. In this flock, the parental, phage-sensitive *Campylobacter* strain and its phage were maintained from the first flock to the next. However, in this second flock, the phage-sensitive *Campylobacter* had largely, but not completely, been replaced by several genotypically unrelated phage-insensitive strains, probably by succession rather than *de novo* development of resistance. In this case, the broiler-house was selected specifically because of the carry-over of strains from the first to the second flock. In practice, however, *Campylobacter* strains do not always persist from one flock to the next within a broiler-house (Petersen and Wedderkopp, 2001; Shreeve *et al.*, 2002). Moreover, the experimental transfer of litter contaminated with excreta from a *Campylobacter*-colonised flock to a new broiler-house did not result in colonisation by genotypically-related strains in chickens reared in the new house, indicating that the incomplete clearance of litter is probably not a critical source for the transfer of infection between subsequent flocks inhabiting the same broiler-house (Payne *et al.,*1999).

In the UK, it is common practice to remove all the litter between flocks and the resulting litter slurries are often negative for the culture of campylobacters, despite positive isolation from birds reared on the same litter. The observation that phage-resistant campylobacters do not emerge as dominant populations, despite their obvious advantage in the presence of phage, and the observation that the majority of infected flocks do not lead to *Campylobacter* strain carry-over would indicate that campylobacters are acquired from the environment and that phage treatment is unlikely to select for the persistence of specific resistant types in the broiler-house environment.

18.2.2 Therapeutic use of bacteriophage to reduce the presence of pathogens in poultry

Published applications involving bacteriophage to treat diseases of poultry include a treatment for *S.* Typhimurium infection that causes considerable morbidity and mortality in young chicks (Berchieri *et al*., 1991). Phage treatments for avian diseases caused by *E. coli* in adult birds cover strains responsible for septicaemia and meningitis (Barrow *et al.*, 1998) and severe respiratory infection (Huff *et al.*, 2003). Most studies of this kind have used a reduction in mortality as a means of assessing success or failure. In relation to food safety, where bacteria such as *Campylobacter* or certain *Salmonella* serovars, are commensal organisms of poultry and cause no obvious pathogenesis in the birds, measuring the success of phage treatment is more challenging. It is very unlikely that bacteriophage would be able to eliminate the target organisms, since predators seldom eliminate their hosts in nature (van den Ende, 1973; Alexander, 1981). However, the efficacy of such treatment will depend on the target microorganism and its impact on human health. Mathematical models of the risk of *Campylobacter* infection in Denmark, for instance, indicate that reductions of two logs or greater in numbers of viable organisms on chicken carcasses could result in a significant reduction (30 times fewer) in the incidence of campylobacteriosis associated with consumption of chicken meals (Rosenquist *et al.*, 2003). Therefore, treatments that do not eliminate, but reduce the numbers below critical thresholds, may have beneficial effects on public health. Strategies that combine microbial treatments with physical and hygiene-control measures could bring about significant reductions in the exposure level of the human population to pathogens.

Practical considerations

The choice of phage is critical to the success of phage treatment, and it should be noted that not all phage that are able to lyse the target bacteria in the laboratory are suitable for practical application (Reynaud *et al.*, 1992). Temperate phage should not be used to reduce the risk of disseminating unfavourable genetic traits through lysogeny and transduction (Schicklmaier and Schmieger, 1995).

Phage have the advantage that they are fairly robust in nature and therefore can simply be added to drinking water and feed, provided that the intended

targets are intestinal bacteria. However, some phage may be sensitive to the low pH encountered in the stomach or proventriculus (Leverentz *et al.*, 2003). This problem can be overcome through the use of antacid or by selection of appropriate low-pH-tolerant phage. Antacids, such as Maalox (aluminium and magnesium hydroxide) or calcium carbonate, have been used to improve the ability of phage to survive low acidity in digestive systems (Smith *et al*., 1987b; Koo *et al.*, 2001). For treating respiratory disease caused by *E. coli* in poultry, phage can be applied via an aerosol spray (Huff *et al.*, 2003). Various systemic diseases have been treated in animals by direct inoculation at the affected site, for example, fish infected with *Ps. plecoglossicida*, which causes bacterial, haemorrhagic, ascitic disease, were treated with phage by intramuscular injection (Park *et al.*, 2000). In order to overcome host immunity issues, it is possible to adapt phage by serial passage to select phage mutants able to survive for longer periods in the circulatory system than parental strains (Merril *et al.*, 1996). When inoculating phage into the circulatory system, it is important that the phage stocks are free of debris from bacterial lysis, which, typically, contains toxins (Merril *et al.*, 1996).

For treating avian disease, the best outcomes are likely to be achieved when phage are applied as soon as symptoms develop. However, the strategy may vary if the purpose is to control an enteric pathogen entering the food chain. In this respect, the point in the poultry rearing cycle at which phage are applied may be critical to success. If the strategy is to overwhelm the bacteria with phage, then administration two to three days before slaughter would reduce the chance of resistance developing. If, however, active phage replication is required, then there will be a prerequisite delay to allow phage to proliferate before the impact on bacterial host numbers. The lead-time must account for phage absorption rates, phage replication rates, the inherent dilution factors associated with the intestinal contents and the transit time of the gut. These processes may be estimated from model data, but the estimates will require validation in practice.

Quality control may be necessary to ensure that the treatment phage can be distinguished from wild type phage and that the phage recoverable from treated birds are the same as those administered. Phage are mutable and can evolve with their host, so the efficacy of stocks must be checked. Phage are also frequent and ubiquitous in the environment, so that contamination of stocks can easily occur. The frequency at which *Campylobacter* phage are isolated from conventional broiler-chicken caecal contents (those that can be propagated on the universal propagating strain *C. jejuni* NCTC 12662, PT14) has been estimated to be 17% in the UK (Atterbury, 2003). The frequency observed in extensively reared birds (organic and free-range flocks) that are exposed to the environment is significantly higher at 50% (A. El-Shibiny, personal communication). The frequency of phage that are specific to other pathogens in poultry intestines is completely unknown, although one report aiming to evaluate phage as faecal indicators showed the incidence of F+ RNA coliphage, somatic coliphage and *Salmonella* phage from chicken breast meat to be 100, 69 and 65%, respectively (Hsu *et al.*, 2002). F+ RNA coliphage are particularly prevalent in chickens, with

one of the highest titres recorded in the survey published by Calci *et al*. (1998). Being able to track particular phage, in order to evaluate treatment success, may not be trivial. In our laboratory, we have designed PCR primers to amplify diagnostic genomic sequences present in specific *Campylobacter*-phage, as a simple assay to be used in experimental systems. Another important quality control issue is that older phage stocks may become less effective, despite retaining high titres in laboratory tests (Weld *et al.*, 2004). Due to the highly specific nature of phage, it has been suggested that they be applied as a mixture or 'cocktail' to cover a broader range of hosts (Kudva *et al*., 1999; Sklar and Joerger, 2001). This tactic will assist in the efficacy of the phage preparation, but will require that the individual components are produced and tested individually, to ensure their contribution to the host range of the target bacterium.

Phage treatment to reduce the presence of Campylobacter

Campylobacter is an obvious target for phage therapy, because of the magnitude of the problem, with more than 80% of birds in the UK harbouring these organisms as a part of their intestinal flora (Newell and Wagenaar, 2000; Corry and Atabay, 2001). The fact that the bacteria are present in the intestines of poultry at very high densities, ranging between logio 4 and logio 8 (Rudi *et al.*, 2004), is a factor that makes phage treatment feasible.

To test the efficacy of phage therapy against *Campylobacter*, it is first necessary to design and evaluate experimental models of *Campylobacter* infection in chickens (Newell and Wagenaar, 2000). We have, in our laboratory, tested candidate bacteriophage isolates from broiler chickens for their efficacy *in vitro* prior to use in experimental birds (Loc-Carrillo *et al.*, 2004). Bacteriophage were administered to the colonised birds at three different doses in an antacid suspension. The reduction in caecal numbers of *C. jejuni* varied with the phage from logio 2 to logio 5 per g of caecal content, compared to controls. By way of comparison, a *Campylobacter* bacteriophage isolated from poultry meat was found to ineffective in a similar trial. Phage-resistant campylobacters were isolated at a relatively low frequency (less than 4%) following treatment, and these resistant strains were compromised in their ability to colonise experimental birds, rapidly reverting back to the sensitive phenotype in the absence of phage. In contrast, phage resistance was maintained as a stable phenotype *in vitro*. Optimisation of dose and selection of appropriate phage were found to be the key elements in the use of phage therapy to reduce campylobacters in broiler chickens.

Wagenaar *et al.* (2001) reported the use of the *Campylobacter* typing phage $\phi 2$ to prevent as well as reduce *Campylobacter* colonisation of broiler chickens. The administration of phage $\phi 2$ resulted in a 3-logio decline in caecal counts of *C. jejuni*. Preventative phage treatment delayed the onset of *C. jejuni* colonisation, and the peak titres remained 2-logio lower than the controls. In both applications, the colony forming units and phage forming units rose and fell over time, and were out of phase with each other, which is typical of predator-prey populations in nature.

Phage treatment to reduce the presence of Salmonella
Phage treatment of chickens was carried out by Sklar and Joerger (2001) in an attempt to reduce levels of colonisation by *S.* Enteritidis. Modest reductions of between 0.3 and 1.3 orders of magnitude were observed in test birds, compared to controls. A selection of 13 different phage were evaluated and some of these were administered with the feed, as this appeared to be the best way to dose the birds.

18.2.3 Use of bacteriophage as a disinfection agent for poultry meat

Bacteriophage can be readily isolated from poultry carcases (Hsu *et al.*, 2002; Atterbury *et al.*, 2003a), so their use as a disinfection agent adds little to what is already present. Bacteriophage have been applied successfully under experimental conditions as a decontamination technique to reduce the presence of *C. jejuni* and *S.* Enteriditis on poultry meat (Atterbury e*t al.*, 2003b; Goode *et al.*, 2003). The most effective treatments involved the use of high doses of phage. Campylobacters are generally believed to be unable to multiply under refrigeration conditions, so the effects are likely to be limited to 'lysis from without' or prevention of re-growth, once suitable conditions for growth are provided, i.e. when contaminated food is consumed. It should also be noted that, on naturally-contaminated carcasses, the bacteria may show different survival kinetics to those in the experimental models described, but this has still to be evaluated. *Salmonella* phage have been used in a similar way on contaminated surfaces. *Salmonella* phage 'cocktails' lytic to 232 of 245 *Salmonella* phage types were found to reduce *Salmonella* contamination to undetectable levels after 48 h. In addition, spraying the same phage preparation on experimentally-contaminated chicken carcasses reduced the numbers of *Salmonella* by approximately 1,000-fold, compared to controls (Chighladze *et al.*, 2001). Experiments with pork adipose tissue have shown that phage may also be used to extend the storage quality of meat by controlling *Brochothrix thermosphacta* (Greer and Dilts, 2002), although results with a cocktail of *Pseudomonas* bacteriophage were less encouraging in the control of beef spoilage (Greer and Dilts, 1990).

18.3 Bacteriocins

Bacteriocins are a heterogeneous group of peptides produced by certain types of bacteria that are active against other (often closely related) bacterial strains. Whilst their use as food preservatives is well established, the use of bacteriocins in live farm animals is at an early stage of development. Bacteriocin production and activity have been demonstrated under laboratory conditions, but not *in vivo;* thus, their usefulness in the intestinal tract is not yet proved (Portrait *et al.*, 2000). However, models of the digestive system have indicated the potential for bacteriocins to survive and be active in the intestine, reviewed by Joerger (2003).

18.3.1 Characteristics of bacteriocins

Bacteriocins were probably discovered around the same time as bacteriophage, but were first described by Gratia in 1925 as filtrates of *E. coli* that inhibited the growth of another strain of the same species. Bacteriocins are produced by both Gram-positive and Gram-negative bacteria, and were the focus of much research during the 1940s and 1950s, reviewed by Gratia (2000). Those produced by the lactic acid bacteria (LAB) are constituents of fermented milk products that have been consumed since ancient times and are therefore considered to be natural and safe (Cleveland *et al.*, 2001). Perhaps the best known of these is nisin, which is in common use as a preservative in the food industry (Abee *et al.*, 1995). Nisin has a long history of use since its discovery in 1928 and recovery from *Lactococcus lactis* (Hurst, 1967).

Bacteriocins are variable in their spectrum of activity, with examples exhibiting narrow host ranges while others will inhibit the growth of a broad range of bacterial species. They are characteristically bacteriocidal and require the presence of a biologically-active protein moiety. The genes encoding bacteriocin production can be chromosomally located or on mobile genetic elements, such as plasmids or transposons, reviewed by Cleveland *et al*. (2001). Bacteriocins are often described using the genus or species designation of the bacterium that produces them (e.g. staphylococcins, colicins, etc), and are classified according to their molecular weight. Some are small (< 40 amino acids), whereas others are large, with molecular weights exceeding 90 000 Da.

The modes of action of the bacteriocins are variable; they may inhibit cell-wall formation, possess nuclease activity or they may cause pores to form in cell membranes. Bacteriocins are often, but by no means universally, heat-stable (15 min. at 100 ºC), but, as proteins, they can be sensitive to proteolytic enzymes. Proteolysis can, of course, be an obstacle, if their intended use is as a feed component in order to reduce intestinal bacteria in live animals. Unless protected, the bacteriocin will be degraded by proteolic digestive enzymes before affecting the target bacteria. Bacteria that produce a bacteriocin are always immune to the bacteriocin by the production of an immunity protein. Genes that encode these proteins are often located in close proximity to those responsible for bacteriocin synthesis (Siegers and Entian, 1995). The exact mechanisms of immunity are complex and, as yet, poorly understood, reviewed by Cleveland (2001).

18.3.2 Use of bacteriocin-producing bacteria to inhibit pathogens

Audisio *et al.* (2000) were able to demonstrate that an *E. faecium* isolate (J96) from a healthy free-range chicken could inhibit the growth of *S.* Pullorum, due to its production of lactic acid and bacteriocin. *In vivo* assays indicated that this strain could protect newly-hatched chicks from challenge with *S.* Pullorum, but it was unsuccessful in treating birds already infected with the bacterium. Laukova *et al*. (2003) demonstrated that an enterocin A-producing strain of *E. faecium* could reduce numbers of *S.* Dusseldorf in the caecal contents of

gnotobiotic Japanese quails. Smith and Huggins (1977) showed that treatment of experimental *E. coli* infections in mice with colicine V favourably influenced the course of the disease. A beneficial effect was noted, even when treatment was delayed until the mice were visibly ill.

It is important to remember that, despite the beneficial aspects of bacteriocins, one group, the colicins, are encoded on plasmids and are strong indicators of bacterial pathogenicity for *E. coli* strains in chickens and other animals (Blanco *et al.*, 1997; Gibbs *et al.*, 2003). Although the bacteriocins themselves are not thought to be involved in pathogenicity, selection of bacterial strains purely on the basis of bacteriocin production would not be advisable. Clearly, extensive evaluation of the pathogenic potential of any bacteriocin-producing strain would be required, if it were to be administered to poultry as a treatment to reduce the presence of pathogens. In addition, other factors, such as the ability to colonise the intestine in competition with other bacteria, would also need to be considered.

18.3.3 Use of bacteriocins to inhibit *Campylobacter*

Antagonistic activities of several bacteria have been demonstrated against the growth of campylobacters (Humphrey *et al.*, 1989; Schoeni and Doyle, 1992; Chaveerach *et al.*, 2004), although it is often unclear whether these activities are due to bacteriocins or to production of metabolites, such as organic acids, or a combination of factors. However, the *Campylobacter*-antagonistic bacteriocins produced by *Bacillus circulans* and *Paenibacillus polymixa* have been characterised (Stern *et al.*, 2003) and crude preparations mixed with feed have successfully reduced or even prevented the colonisation of chickens by *C. jejuni* (Svetoch *et al.*, 2003).

18.3.4 Use of bacteriocins in poultry processing

There are numerous examples of bacteriocins that have been applied as a means of decontaminating poultry meat during processing. For example, nisin can be added to scald water to reduce *Listeria* (Mahadeo and Tatini, 1994). Natrajan and Sheldon (2000) demonstrated the inhibitory effect of nisin on *S.* Typhimurium on poultry skin, using protein- and polysaccharide-based films containing a nisin formulation. Generally, when bacteriocins are used to preserve food, they are used in conjunction with other factors (hurdle technology), such as high or low temperature, detergents, EDTA or the use of modified atmospheres, reviewed by Cleveland *et al*. (2001) and Ross *et al*. (2002). However, it should be noted that meat components can sometimes inhibit bacteriocin activity and any potential application should be validated for the processes and products to which the bacteriocin is to be applied. The fact that naturally-contaminated food may give greater protection to pathogenic bacteria than experimentally-contaminated food should also be considered.

18.4 Regulatory issues

There are requirements for the use of naturally-occurring antimicrobial substances, such as bacteriocins, in food preservation. The toxicology data must be acceptable to recognised authorities. The bacteriocins must not have any deleterious effect on any of the organoleptic properties of the foods on which they are to be used. The form in which the bacteriocin is used must be economic, since the cost of using purified bacteriocins can be prohibitive. The bacteriocin must be stable during storage and, if the activity depends upon a residual, it must be sufficiently stable to cover the shelf-life of the food at effective and probably low concentrations. The bacteriocin should have no medical use. Nisin is the only bacteriocin to have 'generally regarded as safe' (GRAS) status, since it has been approved in 40 countries with a history of use of more than 50 years (Cleveland *et al.*, 2001). For a new bacteriocin to obtain GRAS status, it must be chemically identified and characterised, and its efficacy proved. Details of the manufacturing process, quality control and toxicological data are required. In practice, this has meant that bacteriocins with good food preservation potential, such as pediocin AcH, cannot be used in food at present, although the pediocin has been found to control *Listeria* on raw chicken (Goff *et al.*, 1996). Regulations regarding the use of bacteriophage have not yet been formulated in relation to their therapeutic potential, but first consent for experimental use of bacteriophage to decontaminate food processing plants from *L. monocytogenes* has been given to a US phage company by the US Environmental Protection Agency. Application to the US Food and Drug Administration has been made for the use of bacteriophage as antimicrobial agents on foods, including fresh meat, meat products, fresh poultry and poultry products.

18.5 Public acceptability of the technology

Bacteriocins are perceived as being safe, a natural part of fermented foods that have been consumed unknowingly for many centuries, and are therefore acceptable to the public. The reaction of the public to the use of bacteriophage in food is largely untested. To address this issue, we have organised a focus-group study to gauge public acceptability of phage intervention. In general, the idea of phage intervention therapy was well received by volunteers drawn from the public (for a summary see: http://www.defra.gov.uk/Science/LINK/publications/newsletters/foodlink/FoodLINK_Issue47.pdf). There was a general desire for further research, but, at the end of the proceedings, only one of 23 participants retained concerns over the use of bacteriophage for reducing the incidence of campylobacters in chickens. Key findings were the public preference for labelling the treated product and the opinion that treated foods may be worth a premium. The results of this focus-group study could be of value in relation to other bacteriophage applications involving food production.

18.6 Future trends

Most of the work described in this chapter relates to future possibilities that have potential to reduce the incidence of pathogens in poultry, using bacteriophage- or bacteriocin-producing bacteria. Another group of bacterial predators worthy of mention are a group of parasitic bacteria known as *Bdellovibrio*, which are Gram-negative bacteria that prey on other Gram-negative bacteria. Bdellovibrios have been proposed as potential agents for biological control of pathogenic bacteria, based on laboratory experiments (Jackson and Whiting, 1992; Fratamico and Whiting, 1995). However, the demonstration of effective control *in vivo* has proved more challenging, probably due to high densities of non-prey species and particulate matter that may have a decoy effect (Wilkinson, 2001).

In addition to antimicrobial treatments using microbes, antimicrobial peptides that are not of microbial origin could be exploited by genetic manipulation to enable their production in microbes. Further, the use of bacteriophage-derived enzymes (lysins), produced by genetically modified bacteria, may also be possible, but technically challenging. A successful example of this is the production of murein hydrolase, an endolysin from bacteriophage ϕ3626 that attacks *Clostridium perfringens*. *Cl. perfringens* produces an enterotoxin that can cause foodborne disease and is responsible for severe economic losses in chicken production, as it the aetiological agent responsible for necrotic enteritis. The ϕ3626 endolysin was expressed in *E. coli* and shown to be active against 48 different strains of *Cl. perfringens* (Zimmer *et al.*, 2002). The structures and actions of phage enzymes may provide data allowing the development of synthetic therapeutics (Bernhardt *et al.*, 2002). Phage may also be modified to deliver specific toxins to infecting bacteria (Westwater *et al.*, 2003). Genetic modification of strains to produce bacteriocin is one area where preliminary reports are encouraging. The inhibition of *S.* Typhimurium in the chicken intestinal tract by a transformed avirulent avian *E. coli*, with a plasmid coding for the production of microcin 24, was demonstrated by Wooley *et al.* (1999). Similarly, it has been proposed to engineer avirulent bacteria to produce the antimicrobial peptides produced by many eukaryotic organisms, called defensins. However, it is becoming apparent that the role of defensins is not restricted to antibacterial activity. These proteins have wider antimicrobial properties and can interact with immune regulatory components.

The development of new microbial treatments for poultry is beginning to result in feasible alternatives to conventional antimicrobials. The use of biotechnological tools may accelerate their development, but the public desire for more 'natural' food should not be ignored. However, both bacteriophage and bacteriocins provide the possibility of novel, acceptable solutions to the problems of microbiological safety in the poultry industry.

18.7 Sources of further information and advice

For more information about the history, biology and types of bacteriophage, the book by Adams (1959) is probably an excellent starting point. For up-to-date information, the website of The Evergreen State College, USA (http://www.evergreen.edu/phage/home.htm) is an excellent resource and provides many useful links. The ASM phage group (http://www.asm.org) is another useful resource for the latest phage research. Most fundamental aspects of bacteriocins are covered by Cleveland *et al.* (2001) and their wider use in food preservation by Ross *et al*. (2002).

18.8 References

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19

Irradiation of poultry meat

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19.1 Introduction

Irradiation is one of the most effective intervention strategies for ensuring the microbiological safety of meat and meat products. Poultry meat and eggs have been recognised as major sources of foodborne infections and a high proportion of raw poultry meat products may be contaminated with pathogens such as *Salmonella* and *Campylobacter.* Therefore, radiation treatment of poultry meat, with its potential public health benefits, was being considered, even in the early 1960s (Mossel, 1966; Thornley, 1966). In practice, however, there has been only limited use of the process so far and this is due to a number of factors. The main one is psychological: the weakest aspect of food irradiation is its name, which, unjustly, has connotations of radioactivity and nuclear technology. This has resulted in a low level of acceptance in consumer surveys and a degree of political pressure from some consumer groups and anti-nuclear movements. Consequently, governments and/or regulatory agencies have tended to be over-cautious and overly restrictive in allowing the use of food irradiation (Mossel and Drake, 1990). In addition, the economics of the process depend heavily on the scale of its application and on opportunities to export treated products. Under such circumstances, it is no wonder that the food industry has not made much use of the available treatment options in existing regulations and has been reluctant to pioneer either implementation of the process or education of the public. On the other hand, there has been support from food scientists who have developed and publicised food irradiation (Diehl, 1995) and, particularly, from the Food and Agriculture Organisation (FAO) of the United Nations, the International Atomic Energy Agency (IAEA) and the World Health Organisation (WHO). Over recent decades, these organisations have contributed much towards a better

understanding and acceptance of the process, leading to significant developments and renewed interest in food irradiation, which make it reasonable in this book to consider the more important facts about irradiation of poultry meat.

The main topics to be covered are some basic features of ionising radiation and its technology, the biological effects of the treatment and the radiation sensitivity of pathogenic bacteria that may contaminate poultry meat. Also included are the dose requirements and performance criteria for radiation decontamination of food and factors limiting the doses that can be used. In addition, cost/benefit and wholesomeness aspects of irradiated food, the regulatory status of the process and global developments, emerging applications and future trends will be described briefly.

19.2 Principles of food irradiation and relevant properties of ionising radiation

Food irradiation is a process whereby food is exposed to ionising radiation. Those forms seen as appropriate for food processing are limited to high-energy photons (gamma rays of the radionuclide ^{60}Co, and, to a much lesser extent, ^{137}Cs), X-rays generated by machine, with energies up to 5 MeV, and accelerated electrons, with energies up to 10 MeV. High-energy electron beams are produced by an electron-accelerating machine. X-ray production starts with high-energy electrons in a machine set to X-ray mode, which converts electron energy to electromagnetic X-rays, called 'Bremsstrahlung', by bombarding a heavy metal 'target' with the accelerated electrons. These types of radiation are chosen because they:

- produce the desired preservative effects in food
- do not induce radioactivity in either foods or packaging materials
- are available to a degree and at a cost that allows commercial use of the irradiation process (Farkas, 2004a).

Accelerated electrons have low penetrative capability and the depth-limit for 10 MeV electrons is only 3.9 cm in high-moisture food (or material with an equivalent water content). Gamma rays and X-rays have greater penetrating powers and can be used to treat food, even in pallet-size containers. Except for the differences in penetration, the effects of electromagnetic radiation and electrons are equivalent for treating food.

The energy absorbed by the substance irradiated is measured as the absorbed radiation dose. The relevant SI unit is the gray (Gy), which is defined as 1 joule per kg of product. Doses used in food irradiation are usually expressed in kilograys (kGy). In the earlier literature, the rad was used (1 Gy = 100 rad). For treatment purposes, the D_{10} value is the radiation dose required to reduce the number of viable microorganisms present by a factor of 10 (one log unit).

The potential applications of ionising radiation in food processing are based mainly on the fact that ionising radiation effectively damages cellular DNA, so

that living cells of microorganisms, insect gametes and plant meristems are inactivated or prevented from multiplying, which results in various preservative effects that are a function of the absorbed dose. The basic mechanism of microbial inactivation is believed to involve damage to nucleic acids, either directly or indirectly, from oxidative free radicals arising from the radiolytic breakdown of water molecules.

Differences in radiation sensitivity among microorganisms are related to variations in the chemical and physical structure of microbial cells, and in their ability to recover from radiation injury. Therefore, the absorbed radiation dose required to control microorganisms in foods varies with the sensitivity of the particular species present and the number of cells in each case. In addition, several environmental factors, such as medium composition, moisture content, treatment temperature, fresh or frozen state of the food and presence of oxygen can influence radiation sensitivity, i.e. the proportion of vegetative cells that will be killed by an absorbed radiation dose.

Bacterial spores are generally more radiation-resistant than vegetative cells (in line with their tolerance to other antimicrobial factors). The D_{10} values for spore populations are in the range 1.2–4.3 kGy, depending on species and strain, but with little environmental influence (WHO, 1999). In contrast, the radiation sensitivity of many moulds is of the same order of magnitude as that of vegetative bacteria. However, fungi with melanised hyphae have a radiation resistance comparable to that of bacterial spores (Saleh *et al.*, 1988). Yeasts are as resistant as the more resistant types of vegetative bacteria, while viruses on the other hand, are highly radiation resistant (WHO, 1999).

It is an important observation from data in the literature that bacteria 'surviving' doses of ionising radiation become more sensitive to heat (e.g. Thayer *et al.*, 1991; Grant and Patterson, 1995; Thayer, 1995) and other types of environmental stress (Farkas, 1994; Farkas *et al.*, 1995).

In many ways, irradiation is the ultimate 'minimal process'. Radiation-induced chemical changes in foods are minimal (Thayer, 1990) and the treatment causes virtually no temperature increase in the product. The treatment can be applied to frozen commodities without prior thawing and it will penetrate packaging materials, including those that cannot withstand heat. This means that irradiation can be used as a terminal treatment in processing, even after packaging, thus avoiding re-contamination of the product. By utilising a treatment with no adverse effect on product quality and combining it with the Hazard Analysis Critical Control Point (HACCP) approach to risk management, irradiation offers a physical CCP for improving the safety of many fresh or mildly-processed foods (Molins and Motarjemi, 1997).

19.3 Principal types of radiation source

A typical gamma-irradiation plant (Klinger and Lapidot, 1993) uses a Cobalt-60 source that is composed of a rectangular or cylindrical array of multiple ^{60}Co

elements, doubly encapsulated in stainless steel holders. The chamber (the area of an intense gamma field surrounding the source) is isolated from the environment by heavy concrete shielding. The irradiation chamber also contains a pool of deionised water that is used for storage of the source when it is not operational. A layer of water, 3.5 m deep, above the source provides further necessary shielding. The concrete shielding contains passages, usually in the form of labyrinths that prevent the escape of radiation from the chamber, but allow the product to be moved in and out as required. There is also a properly controlled entrance and exit for operating personnel.

Electron irradiation and X-ray facilities are particularly suited to high-throughput production lines, because their dose rate is by two to four log units higher than that of gamma irradiators. Unlike a radionuclide facility, these machines can be turned on and shut off electronically. Aspects such as the radiation shielding, product-conveyor system and electronic control mechanism can be designed to meet specific operating conditions.

19.4 The role of irradiation in decontaminating fresh or frozen poultry meat

Among the possible decontamination processes, a radiation treatment termed 'radicidation' (Wilkinson and Gould, 1996) is the most effective process for inactivating non-sporeforming pathogenic bacteria. As the Food and Environmental Protection Section of the Joint FAO/IAEA Division correctly states: 'Irradiation, similar to thermal pasteurization of liquid food, e.g. milk, can ensure hygienic quality of more solid food, e.g. poultry and red meat, seafood or spices, without changing significantly their qualities' (IAEA, 1996). The Proceedings of the International Committee of the World Association of Veterinary Food Hygienists (WAVFH, 1967) states that 'for frozen meat and poultry, irradiation is at present the only process that secures reliable decontamination without changing the character or the wholesomeness of the food'. The statement is still valid. This recognition of the process is also reflected in the first 'golden rule' for safe food preparation from the Food Safety Unit of the WHO: 'While many foods, such as fruits and vegetables, are best in their natural state, others are not safe unless they have been processed. For example, always buy pasteurized as opposed to raw milk and, if you have a choice, select fresh or frozen poultry treated with ionizing radiation' (WHO, n.d.).

The non-sporeforming pathogens of principal concern in relation to meat and poultry are relatively radiation-sensitive, as shown in Table 19.1 (Farkas, 2004b), which gives a range of D_{10} values obtained from a large number of publications.

Thayer (1996) constructed a model for the efficacy of gamma irradiation and predicted the responses of *Salmonella* Typhimurium, when present in vacuum-packaged, mechanically-deboned chicken meat and subjected to treatment temperatures of −20 to +20 °C and absorbed radiation doses of 0 to 3.6 kGy. In the context of its 'Pathogen Modeling Program', the United States Department

Table 19.1 D_{10}-values (kGy) of some non-sporeforming bacteria (Farkas, 2004b)

Bacteria	Non-frozen food	Frozen food
Vibrio spp.	0.02–0.14	0.04–0.44
Yersinia enterocolitica	0.04–0.21	0.20–0.39
Campylobacter jejuni	0.08–0.20	0.18–0.32
Aeromonas hydrophila	0.11–0.19	0.21–0.34
Shigella spp.	0.22–0.40	0.22–0.41
Escherichia coli (incl. O157:H7)	0.24–0.43	0.30–0.98
Staphylococcus aureus	0.26–0.57	0.29–0.45
Salmonella serotypes	0.18–0.92	0.37–1.28
Listeria monocytogenes	0.20–1.0	0.52–1.4

of Agriculture (USDA) Eastern Regional Research Center has made predictive models available cost-free, both for *S.* Typhimurium and normal flora on chicken meat, at http://www.arserrc.gov/mfs/irradiation_models.htm.

19.5 Factors influencing the technological feasibility of irradiating poultry meat

19.5.1 Dose requirement, performance criteria and dose-limiting factors

The feasibility of being able to treat a particular food with ionising radiation depends on how much irradiation the food can withstand without showing adverse effects on product quality, and how much benefit is obtained from acceptable and cost-effective doses.

Eventual changes in the organoleptic quality of the product are dose-dependent and increase in intensity and detectability as the dose increases. Threshold doses for some foods of animal origin in relation to organoleptically detectable 'off'-flavours, when irradiated at 5–10 ºC, are shown in Table 19.2 (Sudarmadji and Urbain, 1972). The threshold dose is at least twice as high for frozen poultry meat as it is for chilled products. Subtle changes in odour,

Table 19.2 Threshold doses for an organoleptically detectable 'off-flavour' in some foods of animal origin (Sudarmadji and Urbain, 1972)

Food	Threshold dose (kGy)
Turkey	1.50
Pork	1.75
Beef	2.5
Chicken	2.5
Shrimp	2.5
Frog	4.0
Lamb	6.25

Note: Irradiated at 5–10 ºC.

detectable only by a trained sensory panel, usually dissipate with time after irradiation, and especially during and after cooking. In practice, the recommended doses for radiation processing are 1.5–2.5 kGy for chilled poultry, and 3–5 kGy for frozen items (Wilkinson and Gould, 1996). These doses are adequate to eliminate *Salmonella* and other non-sporeforming pathogens associated with poultry (Thornley, 1963; Mulder, 1982; Kampelmacher, 1983; Lambert and Maxcy, 1984; Patterson, 1988; Huhtanen *et al.*, 1989; Giddings and Marcotte, 1991; Thayer and Boyd, 1991). According to Table 19.1, the radiation doses that are acceptable from a sensory viewpoint can reduce viable counts of the most resistant vegetative pathogens on fresh or frozen poultry by more than 99%. As illustrated by the data of Faw and Mei (1987), such treatment can extend the microbiological shelf-life of chilled poultry two or three-fold compared with untreated samples (Table 19.3). During the early years of research and development on irradiation of poultry, the emphasis was almost exclusively on extending the shelf-life of raw, chilled products (Proctor *et al.* 1956; Idziak and Incze, 1968; Kiss and Farkas, 1972). Guerzoni *et al.* (1993) attempted to model mathematically the dynamics of bacterial spoilage in untreated and irradiated poultry meat.

Authorisation for irradiation of poultry in the USA allows only oxygen-permeable packaging to be used (see Section 19.10.1). Since some poultry products are now packaged under modified atmospheres in order to extend shelf-life, Thayer and Boyd (1999) investigated the combined effect of gamma irradiation and modified atmosphere packaging (MAP) for controlling or eliminating *Listeria monocytogenes*, when inoculated into ground turkey meat. The results suggest that any *Listeria* surviving a dose of 2.5 kGy could be

Table 19.3 Effects of irradiation on total viable counts (log cfu per cm^2) for chicken carcasses stored at 1.6 and 4.4 ºC (Faw and Mei, 1987)

	Irradiation dose						
Days stored	Control 1.6ºC	Control 4.4 ºC	1.3 kGy 1.6 ºC	1.3 kGy 4.4 ºC	2.8 kGy 1.6 ºC	2.8 kGy 4.4 ºC	5.6 kGy 4.4 ºC
1	4.30	3.08				<2.0	2.0
4	4.88	4.81					
7	6.11					<2.0	<2.0
9		7.59		3.45			
11	6.89	8.61					
13							2.79
15	8.41		3.82	6.60		5.30	
17	8.41						
19	8.41						
21				8.80	4.00	8.00	
25			6.84		4.91		
27			6.75				
29					5.61		4.40

prevented from multiplying at a mildly abusive storage temperature of 7 ºC in atmospheres containing 50% or more of CO_2. In relation to product spoilage, a study on fresh, minced chicken meat in MAP and treated by electron-beam irradiation showed that increasing the CO_2 concentration was synergistic with the lethal effect of irradiation and resulted in an increased shelf-life, when compared to the effects of CO_2 or irradiation alone (Grandison and Jennings, 1993).

The effects of irradiation on the sensory properties of chilled and frozen chicken were investigated for skinless, boneless breasts (white meat) and leg portions (dark meat) after treatment at a commercial food-irradiation facility in the USA with doses of 1.66 to 2.86 kGy (Hashim *et al.*, 1995). Trained panels found that the odour of raw, irradiated chicken was more 'fresh chickeny', 'bloody' and 'sweet aromatic' than non-irradiated samples. Also, chilled leg meat was significantly darker than controls, following irradiation.

19.5.2 Changes in the microflora of radiation-'pasteurised' chicken

As with any processing treatment that does not aim to sterilise the product, there are characteristic changes in the composition of the surviving microbial community. The effects of irradiating frozen chicken in this respect can be seen in Tables 19.4 and 19.5 (Prachasitthisakdi *et al.*, 1984).

19.5.3 Colour changes and lipid oxidation in irradiated poultry meat and their control

It has been shown (Hanson *et al.*, 1963; Lynch *et al.*, 1991) that a pink colour is produced in fresh poultry breast meat, when it is irradiated. This can be seen in the higher instrumental a^* values (greater redness coordinates in the CIELAB colour system), when skinned, irradiated chicken breasts are compared with non-irradiated controls (Millar *et al.*, 1995). The increased pink colour was stable during refrigerated storage (Nanke *et al.*, 1998) and occurred in mainly

Table 19.4 Effects of irradiation on the microflora of frozen chicken (data from Prachasitthisakdi *et al.*, 1984)

Organisms	Dose				
	0 kGy	1 kGy	2 kGy	3 kGy	4 kGy
			$\log_{10}$ cfu per g		
Mesophiles	6.8	5.8	4.6	4.1	3.6
Psychrotrophs	5.8	5.7	4.0	<2.8	<1.8
Enterobacteriaceae	5.5	<2.8	1.0	0.4	0.4
Lactobacillus spp.	6.0	4.1	4.2	3.1	< 2.8
Streptotoccoci (enterococci)	5.1	3.7	3.9	3.2	>2.0
Stapyhlococcus aureus	4.6	2.2	<−0.5	<−0.5	<−0.5

Table 19.5 Effects of irradiation on the mesophilic and psychrotrophic fractions of the microflora of frozen chicken (data from Prachasitthisakdi *et al.*, 1984)

Type	Genus	Percentage of total colony count* after irradiation				
		Mesophiles at:			Psychrotrophs at:	
		0 kGy	2 kGy	4 kGy	0 kGy	2 kGy
Gram + cocci	*Aerococcus*	—	10	—	—	—
	Micrococcus	28	30	43	—	83
	Staphylococcus	10	—	—	—	—
	Streptococcus (group D) *(Enterococcus)*	3	43	50	—	—
Gram + rods	*Corynebacterium*	19	3	—	19	3
	Lactobacillus	22	—	—	—	—
Gram − rods	*Acinetobacter*	2	—	—	8	—
	Xanthomonas	—	—	—	4	—
	Pseudomonas	—	—	—	46	—
	Kluyvera	—	—	—	5	—
	Hafnia	10	—	—	7	—
	Klebsiella	—	—	—	11	—
	Escherichia coli	4	—	—	—	—
Yeasts		2	5	7		14

* Limit of detection 2–5%.

anoxic conditions. The importance of reducing conditions in the formation of pink haemochromes in cooked white meat was indicated by Cornforth *et al.* (1986).

While studying colour changes in irradiated meats, Nam and Ahn (2002) reported that irradiation of raw, turkey breast meat decreased the oxidation-reduction potential and the *a** values obtained were positively correlated with the radiation dose and amount of CO produced. Therefore, they suggested that the CO-myoglobin complex is the major haeme pigment responsible for the red or pink colour of irradiated turkey breast. The colour of irradiated breast remains in the meat after cooking (Edmark, 2004). Although the pigment is not harmful, it would worry some consumers. Therefore, research has been initiated to find ways of controlling its development.

Irradiation and storage effects on lipid oxidation have been observed only in aerobically-packaged poultry meat. Lipid oxidation (TBARS values) increased with storage and the increase was more apparent in irradiated meat (Nam and Ahn, 2002; Gomes and Silva, 2003). The oxidative free radicals produced by irradiation would have played a role in promoting the lipid oxidation. Chen *et al.* (1999) reported that phenolic antioxidants were effective in reducing lipid oxidation in aerobically-packaged, irradiated turkey patties. Similarly, a pasteurising dose of 3 kGy enhanced both lipid- and cholesterol-oxidation in chilled, mechanically deboned turkey meat held under aerobic packaging conditions. However, the changes could be controlled by the addition of natural antioxidants, such as thyme oil or a combination of α-tocopherol and ascorbic acid (Lebovics *et al.*, 2002). Dietary supplementation with vitamin E and ascorbate reduced the yield of radiation-induced, off-odour volatiles (Patterson and Stevenson, 1995). Diets containing >200 IU of dl-α-tocopheryl acetate per kg decreased lipid oxidation in irradiated, raw turkey meat (Ahn *et al.*, 1997).

Suitable changes in the packaging method used would be expected to minimise any quality defects in irradiated meats. The use of aerobic conditions for irradiation and a part of the subsequent storage period (one to three days) may allow S-containing, off-odour volatiles to escape from the meat, and such conditions may also be effective in reducing the formation of pink colour. Then, re-packaging the meat under vacuum for the remaining storage period could minimise lipid oxidation during storage (Nam and Ahn, 2003). However, this double-packaging process involves extra cost, labour and time, but not any chemical additives.

It is noteworthy that, in consumer acceptance studies reported by Lee *et al.* (2003), consumers preferred the colour of irradiated, raw and cooked turkey meat to non-irradiated controls, because the pink colour of the irradiated meat made it appear fresher. It was concluded that the combined use of aerobic packaging and antioxidants could be recommended to improve consumer acceptance of irradiated poultry meat. According to further work by the same research group at the Iowa State University Food Safety Consortium, it was found that infusion of chicken breast with rosemary extract enabled irradiated meat to maintain its original colour (Edmark, 2004). Rosemary extract is used as

an antioxidant in several food products, preventing lipid oxidation and providing a spicy flavour that blends well with that of chicken.

19.6 Improving the microbiological safety of minimally-processed poultry products

'Minimally-processed' is an equivocal term that is applied to such different products as pre-cut, pre-packaged fresh produce and mildly cooked meals or meal components that can be stored under refrigeration for more than one week. Their safety depends largely on storage temperature and, eventually, on other barriers to microbial growth, either originating from the raw materials themselves or introduced during processing (e.g. reduced pH, reduced water activity or addition of antimicrobials). In uncooked products, both sporing and non-sporing pathogens should be considered as potential hazards. Among the non-sporeformers, psychrotrophic bacteria, such as *Aeromonas hydrophila*, *L. monocytogenes* and *Yersinia enterocolitica*, are the main concern. With mildly cooked, minimally-processed food that is assumed to be free from post-process contamination, the pathogens of greatest concern are those that may survive the cooking process. In general, these are the sporeforming pathogens, in particular, non-proteolytic *Clostridium botulinum* and certain strains of *Bacillus cereus.*

The USDA has established a 'zero tolerance' policy for *L. monocytogenes* in ready-to-eat processed meats and this organism is considered to be a post-processing contaminant (Klima and Montville, 1995). Reductions in, and survival of, *L. monocytogenes* populations (a five-strain mixture) have been investigated by Foong *et al.* (2004). The organism was inoculated into smoked turkey and other ready-to-eat meats, which were then irradiated. The dosage for a 3-log reduction was 1.5 kGy in the case of smoked turkey, with and without a lactate additive, and a 5-log reduction was obtained with 2.5 kGy. A prolonged lag phase was observed for the surviving fraction of the *Listeria* population, after the meats had been irradiated at 2 kGy and stored at 4 ºC. Patterson *et al.* (1993) conducted a similar study with gamma-irradiated, cooked poultry meat and found that the lag phase was extended to 18 days at 6 ºC for meat irradiated at 2.5 kGy, compared to only one day for non-irradiated meat.

A special version of the 'cook-chill' concept is seen in 'sous-vide' foods. Sous-vide, or vacuum cooking, is a process in which raw foods are cooked under controlled conditions of temperature and time in heat-stable, evacuated pouches (Schellekens, 1993). Sous-vide processing uses only moderate heating temperatures (in the range 65–95 ºC) in order to maximise retention of nutritional and organoleptic properties that would be adversely affected by heating at a higher temperature or in the presence of oxygen.

The combined effect of irradiation and sous-vide cooking was investigated for chicken breast meat by Shamsuzzaman *et al.* (1992) with respect to survival and growth of *L. monocytogenes*, shelf-life, thiamin content and sensory quality. Chicken breasts were inoculated with *L. monocytogenes*, vacuum-packaged,

irradiated, using an electron beam, with doses up to 2.9 kGy, and cooked to an internal temperature of 65.6 ºC. This process caused only a 0.35 log reduction in viable count of *L. monocytogenes.* However, combining irradiation with sous-vide cooking yielded a reduction of more than 5.5 log units and the pathogen remained undetectable in the product during an eight-week storage period at 2 ºC. The electron-beam treatment had little effect on odour or flavour, and the thiamin content was reduced by about only 5%. In a second study (Shamsuzzaman *et al.*, 1995), samples of chicken breast meat inoculated with *L. monocytogenes* at *c.* 10^6 cfu per g were cooked under vacuum to 71.1 ºC and then stored at 8 ºC. Sous-vide cooking alone reduced counts of *Listeria* by only 1.5 log units and the survivors multiplied rapidly during storage. Thus, the non-irradiated samples spoiled within two weeks. The combined treatment (3.1 kGy plus sous-vide cooking) reduced *Listeria* to undetectable levels (< 0.18 log cfu per g), without adversely affecting sensory quality. The combined treatment prevented microbial spoilage for at least eight weeks. The loss of thiamin due to the combined treatment varied from 23 to 46%.

No published data have been found on the effects of irradiation, in combination with sous-vide processing, on survival and growth of *Cl. botulinum.* However, several reports in the earlier literature (e.g. Grecz *et al.*, 1967; Ando and Karashimada, 1972) pointed to sub-lethal injury of *Cl. botulinum* spores that survived irradiation and an increased sensitivity to heat treatment. On the basis of these reports, it could be argued that a combination of irradiation and sous-vide cooking might effectively reduce the incidence of *Cl. botulinum* spores and the risk of subsequent toxin production.

19.7 Radiation sterilisation ('radappertisation') of poultry products

The sterilisation (radappertisation) dose used for low acid foods must ensure elimination of the most resistant bacterial pathogen, *Cl. botulinum*. The dose selected for such a purpose is twelve times that required to achieve a 90% reduction in the endospore population. If the product is required to be shelf-stable, then it must be mildly heat-treated (blanched) before irradiation, to inactivate tissue enzymes. Due to the high dose requirement, the radiation treatment must be delivered to a vacuum-packaged, deep-frozen product to avoid flavour changes and nutrient losses (Urbain, 1986). This technology was pioneered mainly by the US Army National Research and Development Center (Josephson, 1983), and the FDA has approved several radappertised items, including shelf-stable, smoked turkey for military forces and astronauts (Thayer, 2001). The microbiological work was carried out by Anellis *et al.* (1977). Cans of chicken meat were inoculated with 10^6 spores of a mixture of 10 strains of *Cl. botulinum* (five type A and five type B strains), then treated by gamma irradiation at −30 ºC and incubated at 30 ºC for six months, after which the cans were examined for swelling, toxin production and recoverable cells of *Cl.*

botulinum. Using extreme-value statistics, the 12-D dose for chicken meat was calculated as 42.7 kGy, with a 5.1 kGy shoulder on the survival curve. Significant progress has also been achieved in South Africa for radiation-sterilised, shelf-stable food products. Here, several such products, including chicken dishes, have been available on the market since the 1990s and they were well received by the South African military forces (Anon, 1996; De Bruyn and De Williers, 1996).

19.8 Cost/benefit aspects of radiation decontamination

Various North-American studies have concluded that commercial radiation processing of poultry would have a treatment cost of 1–3 US cents per pound (Ouwerkerk, 1981; Kunstadt and Beaulieu, 1989), representing a favourable cost/benefit ratio. In a Canadian study (Todd, 1989), the national annual cost of irradiating processed poultry was estimated at Can$18.5 million, while the benefit in terms of reduced human salmonellosis was estimated to be Can$52.7 million. According to Giddings and Marcotte (1990), electron-beam irradiation of frozen blocks of mechanically separated chicken meat (see Section 19.11), with a minimum dose of 3 kGy, cost the equivalent of about one US cent per kg in France, which was considered to be cost-effective.

19.9 Wholesomeness of irradiated food

Wholesomeness (nutritional adequacy, microbiological safety and freedom from toxicological problems) of irradiated food has been carefully evaluated by an unprecedented amount of research and testing over more than 50 years. All the scientifically acceptable evidence from these studies supports the view that irradiated food is entirely safe for human consumption (WHO, 1981, 1994, 1999; Diehl, 1995). Regarding nutritional adequacy, the reduction in level for the most sensitive vitamin, thiamin, is about 10% at doses around 3 kGy (Giddings and Marcotte, 1990), which is no cause for concern. Questions about the microbiological safety of foods pasteurised by ionising radiation have been considered carefully in detailed reviews (Ingram and Farkas, 1977; Farkas, 1989) and no problems have emerged.

Following a publication by Le Tellier and Nawar (1972), it is known that 2-alkylcyclobutanones are produced in trace amounts from the radiolysis of triglycerides. This suggests that 2-dodecylbutanone (2-DCB), formed from palmitic acid, could be used as a marker for irradiated chicken (Stevenson, 1996) (see also Section 19.10). Recent work by Delincee and Pool-Zobel (1998), using the DNA comet assay, claimed that 2-DCB induced DNA strand-breaks in intestinal cells from rodents and humans. This raised the possibility that the compound was a weak genotoxin, causing some consumer groups to assume erroneously that 2-DCB was mutagenic and, therefore, that irradiated foods could

cause cancer. However, reviews of the above results by regulatory agencies (e.g. Health Canada, 2003) showed the claim that 2-DCB was genotoxic was not supported by the experimental evidence. In the most recent study in the USA (Sommers and Schiestl, 2004), 2-DCB did not induce mutations in the *Salmonella* mutagenicity test or cause intra-chromosomal recombination in the yeast, *Saccharomyces cerevisiae*. The absence of genotoxicity in this study, using purified 2-DCB, agrees with the lack of genotoxic or teratogenic activity observed previously in long-term, multi-generation feeding studies on laboratory animals that were given radiation-sterilised poultry containing 2-DCB as a unique radiolytic product (Thayer *et al.*, 1987; WHO, 1994).

19.10 Regulatory aspects of food/poultry meat irradiation

19.10.1 Approvals

On the basis of the report of the FAO/IAEA/WHO Expert Committee on Food Irradiation (WHO, 1981), the Codex Alimentarius Commission established a Codex General Standard for Irradiated Foods and a Codex Recommended International Code of Practice for Radiation Processing of Food. Their revision (CAC, 2003) was adopted in 2003 by the Commission at its twenty-sixth session.

Currently, some 50 countries have granted national clearance for the irradiation of at least one food item or food class. In addition to previous approvals, the US Food and Drug Administration (FDA) has sanctioned the use of irradiation to control foodborne pathogens in poultry (FDA, 1990). The FDA established a maximum dose of 3.0 kGy, while the USDA Food Safety and Inspection Service (FSIS) requires a minimum dose of 1.5 kGy. US law allows only oxygen-permeable packaging to be used for irradiated poultry (Anon., 1992; FSIS, 1992).

An itemised database from the International Consultative Group on Food Irradiation (ICGFI) gives details of national clearances and can be visited on the website: http://www.iaea.org/icgfi/ (Food Irradiation Clearance Database). This data collection lists at least 18 unconditional national clearances for the control of microorganisms on poultry meat by ionising radiation. However, the existence of positive legislation does not necessarily imply commercial utilisation of the process.

Unlike the supporting attitude of the relevant specialist agencies (e.g. WHO, FAO and IAEA), the European Parliament and the Council of the European Union have a much more restrictive approach to legislation on this topic. In 1999, the 'framework' Directive 1999/2/EC (EP, 1999a) was issued to control laws of the Member States concerning foods and food ingredients to be treated by ionising radiation, and Directive 1999/3/EC (EP, 1999b) appeared on the establishment of a Community List of foods and food ingredients that were allowed to be treated. The present category of foodstuffs authorised by the latter Directive for irradiation treatment is 'dried aromatic herbs, spices and vegetable seasonings'. Through Directive 1999/2/EC, the European Commission was

charged with developing a final, positive list of permitted items, up to the end of the year 2000; however, this list has yet to be published. Instead, national regulations in Belgium, France, Italy, Netherlands, Spain and the United Kingdom, which were established before the EC Directive, came into force.

19.10.2 Labelling requirements, process control and methods for detecting irradiation treatment

Proper control of food irradiation should meet the requirements of both food and irradiation technologies. Food irradiation should never be used to replace good manufacturing and hygiene practices, but to complement them. Regulations also specify requirements for record-keeping and labelling. Thus, process control responsibilities are shared by the food producer, the processor and the operator of the irradiation plant. Processing records include details of qualifying products, information on actual processing conditions, detailed dosimetry and product labelling (Giddings and Marcotte, 1990). Most regulations require the international logo/symbol of food irradiation, plus the words 'treated with/by radiation/irradiation' or 'irradiated'.

To improve consumer confidence and assist international trade in irradiated foods, detection methods have been developed (Delincée, 1991). For irradiated food containing bone, electron spin resonance (ESR)-spectroscopy is the detection method of choice. This measures radiation-induced, relatively long-lived free radicals in the hard bony material (Desrosiers and Simic, 1988; Stevenson and Gray, 1989). The presence of 2-DCB has been proposed as a potential indicator for lipid-containing, irradiated foods, including poultry (Murray *et al.*, 1996). The DNA comet assay or gas-chromatographic analyses of radiation-induced hydrocarbons formed from fats can also be used to detect irradiated poultry meat (Cerda, 1998; ECS, 2003; Chung *et al.*, 2004).

19.11 Future trends

There is no scientific reason why irradiation should not be used, if it serves a meaningful purpose. It is estimated that more than 30 countries are currently using the technology, sometimes semi-commercially, for certain foods or food ingredients. The process has been implemented commercially in France since the late 1980s by the Société de Protéines Industrielles (SPI), a subsidiary of the Guyomarc Group, for mechanically separated poultry meat (MSM) (Sadat and Volle, 2000). MSM invariably carries substantially more contamination, including pathogens, than intact carcasses or parts. The SPI employs two-sided electron-beam irradiation of 5.5 cm thick frozen slabs held at −18 ºC, using a minimum radiation dose of 3 kGy and keeping within the 5 kGy maximum dose (Giddings and Marcotte, 1990). As well as eliminating vegetative pathogens, total viable counts are considerably reduced by this means, so that the irradiated MSM will keep longer after thawing, if extended chill-holding is necessary.

In the USA, an irradiation service company (Food Technology Service Inc., Mulberry, FL) has been irradiating poultry since 1996 and at least one regional restaurant chain uses irradiated chicken exclusively (Anon., 2003). A recent survey in the metropolitan Atlanta area to determine consumer attitudes towards irradiated foods, after eating irradiated, ready-to-eat poultry meat, has shown that consumers were more concerned with bacterial contaminants, food additives and pesticide/veterinary drug residues than with irradiation itself (Johnson *et al.*, 2004). The study also indicated that consumers were significantly less concerned about food irradiation in 2003 than in 1993, when a similar study was conducted. The authors concluded that most consumers felt they were not informed about the advantages of the irradiation process and, therefore, with more education and greater exposure to irradiated products, the concerns should diminish.

Most recently, there has been an important development in the USA, where irradiation of fresh ground beef and frozen beef patties, and commercial production and marketing of these products, started in 2000 to eliminate or significantly reduce the incidence of *Escherichia coli* O157:H7 and other hazardous bacteria. Since then, a nationwide distribution of such irradiated foods has become possible and the amount of irradiated ground beef increased to 50 millions lbs in 2003 (Tatiana Rubio-Cabello, Joint FAO/IAEA Division, Vienna, Austria, personal communication). Several USDA agencies are collaborating to make irradiated meat and poultry available for use in the National School Lunch Program in the USA and the USDA-FSIS has developed an educational programme on irradiated meat and poultry (Murano, 2003). Another important step towards wider application of food irradiation in the future is a pending petition by a Coalition of Food Irradiation, submitted to the FDA in 1999 for approval of irradiation for many types of ready-to-eat foods. Health Canada is also considering amendment of its regulation on food irradiation on the basis of a formal request for approval for several types of food, including fresh and frozen poultry.

Even after the earlier pioneering efforts in several European countries, e.g. Netherlands, France, Belgium and the UK, progress in introducing food irradiation into Europe is now lagging behind developments in the USA, due to legislative difficulties and the activities of certain anti-irradiation groups. As Dr M. Satin, former chief of the FAO Agro-Food Industry and Post-Harvest Management Service, has pointed out in one of his papers (Satin, 2002), the situation in Europe is the same as that experienced in the past with heat pasteurisation of milk: ‘Politics and consumer advocacy combined to delay the institution of pasteurisation in Europe long after it was commonplace in North America’. This difference in attitude between the two regions confirms the observation that risk and risk perception are social constructs (Gibney, 2004). However, increasing interest in food irradiation in the USA and some countries of Asia and Latin America are likely to persuade other countries and regions in the future to implement the use of food irradiation for commercial purposes.

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20

Rapid detection and enumeration of pathogens on poultry meat

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20.1 Introduction

The poultry industry is continuously in search of rapid and inexpensive methods for enumerating and identifying microorganisms on poultry products and processing equipment. Traditional microbiological techniques are advantageous, because they have been tested extensively for accuracy and they are accepted by regulatory agencies, both nationally and internationally. However, conventional methods are cumbersome, labor-intensive and costly, and they require long periods of time to yield results. Conventional methods for detection, enumeration, identification and classification of microorganisms are described in texts such as the *Compendium of Methods for the Microbiological Examination of Foods* (Vanderzant and Splittstoesser, 1992), *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC, 1990), the *Bacteriological Analytical Manual* (FDA, 1992), *Standard Methods for the Examination of Dairy Products* (APHA, 1985) and *Modern Food Microbiology* (Jay, 1992).

The time required for microbiological tests to be completed becomes critical when considering the disposition of poultry products that are ready for distribution. Traditional aerobic plate counts (APC) require 48–72 h to conduct (Busta *et al.*, 1984). Detection of specific pathogenic bacteria, such as *Salmonella*, using conventional methods, may require up to seven days (Andrews *et al.*, 1984). Psychrotrophic plate counts need an incubation period of ten days at 7 + 1 ºC (Gilliland *et al.*, 1984), or its equivalent. Since freshly processed poultry should be shipped to market within 24 h of processing (*Prepared Foods*, 1988), poultry products may reach the consumer even before microbiological tests have been conducted.

Recently, numerous product recalls experienced by the poultry industry have clearly pointed to the necessity for rapid testing of products. For example, in the year 2000 in the USA, the US Department of Agriculture (USDA) reported that 18,081,829 lbs of ready-to-eat meat products from 34 companies were recalled due to *Listeria* contamination. This equates to approximately $117,531,888 worth of product. In 2002, the USDA reported recalls of 31,800,000 lbs of ready-to-eat meat from only five companies. The losses to the industry due to recalls were enormous and these figures do not account for human pain and suffering, loss of reputation on the part of the suppliers and costs associated with litigation.

The purpose of this chapter is to describe rapid methods for enumerating and identifying microorganisms that are of importance to the poultry industry. These techniques can be used by microbiologists for both quality control and research. In general, microbiologists working with poultry are concerned with sample collection and preparation, total viable counts, detection and enumeration of pathogenic bacteria, and monitoring of processing plant sanitation.

20.2 Sampling methods for poultry products

The methods commonly used to sample fresh poultry for microbiological analysis include:

1. whole-carcass rinse
2. tissue swab
3. nitro-cellulose membrane lifting
4. excision of tissue for stomaching.

20.2.1 Whole-carcass rinse

Historically, the whole-carcass rinse procedure has been considered the most appropriate method of sampling fresh poultry carcasses, although organisms that are firmly attached to carcass surfaces are not necessarily recovered by this means. Nevertheless, the method is particularly useful in relation to small bacterial populations, such as those of *Salmonella* (Barnes *et al.*, 1973; van Schothorst *et al.*, 1976; Cox *et al.*, 1978). The whole-carcass rinse procedure involves placing a carcass in a sterile polyethylene bag with 100 ml of sterile water and shaking vigorously for 1 min. (Cox *et al.*, 1981). After shaking, microbiological tests are carried out on the rinse fluid. If conducted properly, this procedure is fatiguing, especially if there are large numbers of broiler carcasses to be sampled. Sampling turkeys is even more difficult, because of their large size and the need for greater volumes of sterile water. The numbers of organisms removed from carcasses in this way may vary as a result of exhaustion of laboratory personnel. Therefore, an automated method for rinse-sampling of carcasses has been developed (Dickens *et al.*, 1985, 1986). This apparatus

resembles a paint-shaker onto which six cans for broilers or two large cans for turkeys have been mounted. Bagged carcasses are placed in the cans, the lids of the cans are sealed and the carcasses are shaken mechanically for 1 min. The automated procedure was found to be as effective as shaking by hand for determining APC, counts of Enterobacteriaceae and for recovering *Salmonella* (Dickens *et al.*, 1985, 1986). The advantages are that much less labor is required and, theoretically, error associated with variation in sampling due to operator fatigue should also be less.

20.2.2 Swabbing

The conventional swab technique is likely to remove only a small proportion of the organisms present. It involves rubbing five areas (approximately 50 cm^2) of the food item to be sampled with a pre-wetted, sterile calcium alginate swab. This swab is then placed in 4.5 ml of a sterile solution containing 1% sodium citrate to dissolve the swab and release the organisms (Sveum *et al.*, 1992). One and 0.1 ml portions of the resultant suspension are then used to prepare pour-plates, using plate count agar and/or other suitable media (Sveum *et al.*, 1992). Plates are incubated at 35 ºC for 48 + 3 h and those with 30–300 colonies are counted. This method requires labor to prepare solutions and media, dilute samples, inoculate the plates and count the incubated plates. Forty-eight hours are required to obtain results and the poultry products in question may have been consumed by the time that results are available.

20.2.3 Nitro-cellulose membrane lifting

A nitro-cellulose membrane-lifting technique for removal of *Salmonella* from chicken skin has been developed by Tsai and Slavik (1991). This procedure involves placing an 8 cm^2 piece of chicken skin onto a similar-size area of 0.45 μm nitro-cellulose membrane that has been pre-moistened with phosphate-buffered saline. Filter paper is then placed on the skin and rubbed with a sterile, bent glass rod. The membrane is then transferred onto a solid culture medium that is selective for *Salmonella*. This method was reported to be more effective than conventional swabbing for removing *Salmonella* from chicken skin (Tsai and Slavik, 1991).

20.2.4 Tissue excision

To sample poultry meat and products using the tissue-excision method, a 25 g sample is removed and placed in a sterile blender jar with 225 ml of sterile, phosphate-buffered diluent. The sample is then blended for 2 min., which allows all microbial contaminants present to be recovered. The disadvantage associated with this technique is that labor is required to clean and sterilize the blender jars. In fact, stomaching is an excellent alternative to blending. Stomaching refers to the process of macerating tissue, using a device that pummels the sample

continuously by means of two reciprocating paddles. The procedure for stomaching is similar to that for blending; however, the meat and diluent are placed in sterile, disposable bags instead of blender jars. Labor is reduced by disposing of bags after samples have been stomached, as opposed to washing and sterilizing blender jars. In a study conducted by Emswiler *et al.* (1977), microbial counts obtained by stomaching were similar to those from blending. A comparison of blending versus swabbing for broiler skin was reported by Cox *et al.* (1976).

20.3 Detection methods

20.3.1 Genetic methods

DNA hybridization

Gene-Trak® Systems (Hopkinton, MA, USA) have developed DNA hybridization assays to screen samples for the presence of *Salmonella, Campylobacter, Listeria, E. coli, Staphylococcus aureus* and *Yersinia enterocolitica*. Although each individual procedure differs slightly, a general description of the methods used will be presented.

To conduct DNA hybridization assays, the target bacteria in the sample must be pre-enriched to increase the number of organisms present to a point where they can be detected (Gene Trak, 1991). After pre-enrichment, hybridization assays are conducted. To conduct a *Salmonella* assay, all bacteria in the sample are lysed, using a lysis solution, and the *Salmonella* rRNA target strands are released into the solution. Two DNA probes with different, distinct functions are then added to the solution. Both probes are homologous to unique rRNA sequences in *Salmonella* and the two probes hybridize with adjacent regions of the same target rRNA molecule (Gene Trak, 1991). The capture probe contains a poly dA tail. The purpose of the poly dA tail is to allow the hybrid molecules to be captured onto a solid support that binds to the poly dA tail. The detector probe is labeled at both ends, using fluorescein. This probe binds to the end of the hybrid molecule that is not bound to the solid support. The final hybrid molecule contains a strand of *Salmonella* rRNA that has two probes attached. The hybridization reaction is carried out at 65 ºC (Gene Trak, 1991). The next step in the assay is to capture the hybrid onto a solid support. The support used is a plastic 'dipstick' that is coated with poly dT. The poly dA tail on the hybrid molecule attaches to the poly dT on the dipstick and the molecule is captured.

The remaining step in the assay is to detect the strand of *Salmonella* rRNA that has been captured on the solid support. The detector probe at the other end of the captured hybrid is first treated with a polyclonal anti-fluorescein antibody (anti-Fl) conjugated to the enzyme, horseradish peroxidase (HRP). This conjugate then binds to the flourescein molecules on the detector probe. The complex is allowed to react with a substrate of HRP, hydrogen peroxide, in the presence of a chromogen. A blue color develops in proportion to the amount of enzyme conjugate that is bound to the complex and, thus, is also in proportion to

the amount of *Salmonella* rRNA captured. The reaction is stopped with sulfuric acid and this changes the color that has developed from blue to yellow. The color intensity is measured by determining the absorbance at 450 nm, using a photometer. An absorbance in excess of a pre-determined cut-off value indicates a positive result for the presence of the target organism (Gene Trak, 1991).

Rose *et al.* (1991) compared a DNA hybridization method for the detection of *Salmonella* in meat and poultry products to conventional methods of bacterial identification. The authors reported that the DNA hybridization procedure was more sensitive than the culture methods tested. There were no false-positive or false-negative results using the colorimetric DNA hybridization method. Another DNA hybridization assay used for detection of *E. coli* was evaluated by Hsu *et al.* (1991). Using this assay, the authors were able to detect all 233 strains of *E. coli* tested. Of the 207 non-*E. coli* species tested, only *E. fergusonii* and *Shigella* caused false-positive results. The total false-negative rate was 1.2%, whereas, the false-negative rate for the conventional culture method was 23.4%. These authors reported that the DNA hybridization method was significantly more accurate than conventional methods for the detection of *E. coli* in foods.

Polymerase chain reaction (PCR)

PCR is a method used to amplify a specific, target segment of DNA from a complex mixture of microorganisms, as a means of detecting a particular organism. The reaction synthesizes millions of copies of a specific DNA segment within a few hours. The reaction mixture contains a heat-stable DNA polymerase, free nucleotides and a pair of 'primers' (short DNA sequences complementary to the specific target-DNA sequence), in a total volume of 25–100 μl (Lantz *et al.*, 1994).

The PCR method is based on the repetition of three steps, all conducted in succession, each at a different controlled temperature. Each step requires about 60 sec. to perform. In the first step, the two strands of the target DNA sequences (bacterial DNA) are separated using heat denaturation (Lantz *et al.*, 1994). As the temperature is lowered to the annealing temperature (45–65 ºC), each primer will anneal (hybridize) to only one of the separated strands of bacterial DNA. The primer sequence is determined by the nucleotide sequences flanking the target DNA sequence that is to be amplified (Lantz *et al.*, 1994). The third step in the procedure is the synthesis of the complementary strands at the ends of each primer. The heat-stable DNA polymerase begins to synthesize new target DNA (PCR products), by adding free nucleotides to the primers. The authors state that newly-synthesized PCR products can then serve as templates in the following rounds of amplification. After 30–40 cycles of heat denaturation, annealing and primer extension, target DNA sequences will have been amplified by a factor of 10^9 (Lantz *et al.*, 1994). By this exponential amplification [(PCR product)^n, where n is the number of cycles], it becomes possible to detect a specific DNA region by gel electrophoresis or by a computerized DNA detection system. Lantz *et al.* (1994) observed that, in a few cases, food samples that

contain whole bacteria can be used directly as PCR samples, because the repeated cycles of heat denaturation will release bacterial DNA. However, appropriate sample preparation is required before carrying out a PCR on the majority of food samples, because they can contain substances that inhibit the PCR and/or low numbers of pathogenic bacteria (Lantz *et al.*, 1994).

Loeffelholz *et al.* (1992) reported that PCR had a sensitivity of 97% and a specificity of 99.7%, while traditional culturing had a sensitivity of only 85.7% and a specificity of 100%, when detecting *Chlamydia trachomatis*. PCR is an extremely sensitive and specific method for the detection of pathogenic bacteria in clinical samples. However, for analyzing food samples, this technology is still somewhat difficult to use. It requires extensive training, is expensive and cannot differentiate between DNA derived from live bacterial cells and that from dead cells. Therefore, PCR would not be suitable for analyzing samples of cooked food that originally contained pathogenic bacteria.

20.3.2 Substrate-supporting disc method

ColiComplete™

A simple, rapid method has been developed to determine the presence of coliforms and/or *E. coli* in food samples. The ColiComplete™ kit (BioControl Systems Inc, Bothell, WA, USA) includes discs that are impregnated with X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) and MUG (4-methylumbelliferyl-beta-D-glucuronide). Samples are placed in lauryl sulfate-tryptose (LST) broth and a ColiComplete™ disc is added. The sample is incubated at 35 ºC for 48 h. If coliforms are present in the sample, they will produce β-galactosidase that oxidizes X-Gal to form a blue derivative. If the blue color is observed, this confirms the presence of coliforms. *E. coli* produces β-glucuronidase that will convert the MUG on the disc to a blue, fluorescent compound. Samples showing a blue fluorescence, when held under a short-wave UV light, are regarded as positive for *E. coli*.

Feldsine *et al.* (1993a) compared The ColiComplete substrate-supporting disc (SSD) method for simultaneous confirmation of coliforms and *E. coli* with the AOAC Most Probable Number methods (966.23 and 966.24). The authors found that results were statistically equivalent for all levels of total coliforms in all types of food analyzed, except frozen vegetable and raw nut meat, uninoculated control samples and one lot of pork sausage for which the SSD method produced significantly higher numbers. For *E. coli* determinations, results were statistically equivalent across all samples and levels of contamination for each type of food (Feldsine *et al.*, 1993b).

20.3.3 Immunological methods

Salmonella-Tek™, Listeria-Tek™ and EHEC-Tek™

ELISA procedures have gained much popularity in the last decade as a means of detecting specific populations of pathogenic bacteria. Salmonella-Tek™,

Listeria-Tek™ and EHEC-Tek™ (bioMérieux Vitek Inc, Hazelwood, MO, USA) are popular enzyme assays currently used in the poultry industry.

To detect *Salmonella* on fresh meat with Salmonella-Tek™, samples are pre-enriched for 22–26 h, sub-cultured and incubated for 18–24 h in selenite-cystine broth and tetrathionate broth; they are then sub-cultured in M broth for 6 h, centrifuged, heated for 20 min. in boiling water and cooled. One hundred μl of sample and control fluid is pipetted into each of the wells in a strip-plate that is coated with *Salmonella* monoclonal antibody. In this step, the monoclonal antibody at the base of the well is specific for *Salmonella* and captures any that are present in the sample. With the *Salmonella* captured, each cell is washed three times with a wash solution to remove other bacteria or any material that may interfere with the test. Then, 100 μl of conjugate is added to each well. The conjugate is another antibody that is specific for *Salmonella*. This conjugate binds to that part of the *Salmonella* cell that is not attached to the well and bears an enzyme. The wells are sealed and incubated at 37 ºC for 30 min. Each well is then washed six times with a wash solution to remove extraneous material, before 100 μl of freshly-prepared TMB solution are added and the plates incubated at 20–25 ºC for 30 min. The TMB (tetramethylbenzidine) substrate reacts with the enzyme attached to the conjugate antibody and causes a color change.

After incubation, the color reaction is halted by adding 100 μl of 'stop' solution. The resultant color change is determined by examining each well photometrically at 450 nm. If no *Salmonella* cells are present in the sample and therefore cannot be captured by the antibody in the well, no conjugate binds to them, no TMB substrate binds to the conjugate and no color change occurs. The time needed to complete this procedure is approximately 48–58 h.

The ELISA procedure, using Listeria-Tek™ and EHEC-Tek™, is similar to that for Salmonella-Tek™ except that the sample preparation is different. Using Listeria-Tek™, samples are pre-enriched in UVM (University of Vermont Medium) broth at 30 ºC for 24–26 h, sub-cultured into Fraser Broth, which is incubated at 30 ºC for 24–26 h, heated for 20 min. in boiling water and cooled, before conducting the ELISA assay. The time required for this procedure is approximately 50–54 h. For EHEC-Tek™, 25 g of sample are placed in a solution containing 225 ml of modified EC (*Escherichia coli*) Broth + Novobiocin and shaken while being incubated at 37 ºC for 20–24 h. The culture is then heated in boiling water for 20 min. and cooled before conducting the ELISA assay. The time required for the procedure is approximately 22–26 h.

TECRA® Salmonella Visual Immunoassay and Listeria Visual Immunoassay

TECRA® Salmonella Visual Immunoassay and Listeria Visual Immunoassay (Tecra diagnostics, Roseville, NSW 2069, Australia) are also popular ELISA methods. The ELISA procedures for TECRA® Salmonella Visual Immunoassay and Listeria Visual Immunoassay are similar to those for Salmonella, Listeria and EHEC-Tek™, except that sample preparation is different. To test for *Salmonella*, the procedure for TECRA® Salmonella Visual Immunoassay recommends pre-enrichment of samples for 18–26 h, selective enrichment for 16–20 h and post-

enrichment incubation for 6–8 h, prior to conducting ELISA assays. The total time required for this procedure is 42–56 h. To test for the presence of *Listeria*, the TECRA® Listeria Visual Immunoassay procedure recommends primary enrichment of samples for 24 h and secondary enrichment for 22–24 h prior to conducting ELISA assays. The total time required is 48–50 h. Color changes may be determined manually or automatically, using a reader.

Noah *et al.* (1991) conducted a study to compare the efficiency of Listeria-Tek™ and the TECRA® Listeria assay. In total, 178 samples of processed crabmeat, hot dogs, sausage bisquits, lobster tails, raw onion rings, raw shrimp and lettuce were examined. Using the Bacteriological Analytical Manual (BAM) method, Listeria-Tek™ and TECRA® Listeria assay, the presence of *Listeria* was detected and culturally confirmed in 38, 37 and 40 samples, respectively. The authors concluded that, as rapid screening methods, the Listeria-Tek™ and TECRA® Listeria assays qualify as acceptable alternative methods to the BAM cultural method.

TECRA® OPUS®

The TECRA® OPUS® (TECRA Diagnostics, Roseville, NSW 2069, Australia) is the most highly automated ELISA system available for detecting the presence of many pathogenic bacteria and their toxins. All of the ELISA reactions are conducted within one OPUS® module. The module contains a mixing well, an antibody-coated fiber matrix, a wash port, a substrate well and a well containing an antibody-enzyme conjugate. The module is bar-coded for easy identification of samples. Twenty samples per hour may be tested and a number of different assays can be conducted simultaneously.

After preparing the sample, the assay type is selected, the module is transferred to the instrument and the sample is introduced. The instrument then adds conjugate to the sample and this binds to any target antigens (pathogens) present to form an antibody-conjugate complex. The instrument then pipettes this mixture onto the fiber matrix and any antigen-conjugate complex will be captured by the highly-specific antibodies on the fiber. The 4-methyl umbelliferyl phosphate (MUP) substrate is added through the wash port and drawn across the fiber, washing away any unbound material and producing fluorescence in the presence of the target antigen. The instrument interprets the fluorescence of the sample, using a tungsten-halogen light source. TECRA® OPUS® is extremely easy to use; however, the initial cost of the instrument is high.

Reveal™

Reveal™ test strips (Neogen Corp, Lansing, MI 48912, USA) combine an ELISA sandwich immunoassay with capillary action to provide a one-step screening test for *E. coli* O157:H7 that takes only 20 min. Samples to be analyzed are first pre-enriched for 24 h. A sample of the pre-enrichment culture is then placed in the sample port. The sample is drawn by capillary action through the pad present to the specimen-reaction zone, which contains colloidal gold-labeled antibodies specific for *E. coli* O157:H7. If the organism is present

in the sample, it will complex with the gold-labeled antibodies and migrate through the support until it encounters a binding-reagent zone that includes a second antibody specific for *E. coli* O157:H7. When this occurs, a line appears in the test window due to a concentration of the gold label. The remainder of the sample continues to migrate until it encounters a second binding-reagent zone. Again, this results in the formation of a line, this time in the control window. Regardless of whether or not the sample contains *E. coli* O157:H7, a line will form in the control window, thus indicating that the test is working properly.

Samples to be analyzed for *Salmonella* are placed in Revive™ medium at 35 ºC, which is incubated for 2 h. Revive™ is a medium used to resuscitate any *Salmonella* present in the sample and can be used to aid recovery of injured cells from meat and poultry. After resuscitation, samples are incubated in selenite-cysteine broth at 43 ºC for 18 h. A sample of the culture from selective enrichment is placed in the sample port. The method by which antibody reactions are used to screen for *Salmonella* is the same as that for *E. coli* O157:H7. The test strips are extremely easy to use and comparatively inexpensive.

VIP®

VIP® test strips (BioControl Systems Inc, Bothell, WA, USA) utilize an immuno-precipitate method in which antibodies with high specificity for *E. coli* O157:H7 are bound to a carrier and bound separately to a solid support-matrix. These reagents are configured in a single-use device that will produce a visually determined reaction in the presence of *E. coli* O157:H7. Samples to be analyzed are pre-enriched in modified tryptic-soy broth plus novobiocin for 24 h. A sample (0.1 ml) of the pre-enrichment culture is then placed in the sample port. During the initial hydration phase, *E. coli* O157:H7 will react with an antibody-chromogen complex contained in the device. If the organism is present in the sample, a visible detection line will form in a viewing window across the solid support. The formation of such a line indicates a positive test. Additionally, a control window is provided after the test-line window to ensure that the sample has migrated properly along the solid-support matrix. Formation of a line in the second window indicates that the test is complete. Absence of a control line in the second window indicates an invalid test. The test kit is very easy to use and comparatively inexpensive.

Overall, ELISA test kits are sensitive and inexpensive. However, they are more labor-intensive than other, more automated methods for the detection of target bacteria.

Enzyme-linked immunosorbent assay metal-bead lift

Although ELISA methods for detecting *Salmonella* and *Listeria* are rapid, they require a selective or culture step that takes an additional 18–24 h of incubation before the ELISA procedure can be carried out. A method has been developed to avoid the selective or culture step and this is Dynabeads® anti-Salmonella (Dynal Biotech Inc, Brown Deer, WI, USA). Samples are pre-enriched for 24 h

to increase any populations of *Salmonella*. Dynabeads® are then added to 1 ml of the sample. Dynabeads® are uniform, super-paramagnetic microspheres (2.8 μM in diameter) with affinity-purified, anti-*Salmonella* polyclonal and monoclonal antibodies on their surface. If *Salmonella* is present in the sample, it is captured by the anti-*Salmonella* antibody. A magnet can then be used to attract the metal beads with the captured *Salmonella* to the side of the vial. While the metal beads are held to the side of the vial, the vial is washed to remove extraneous material. The beads can then be cultured by traditional plating methods or rapid methods may be used, such as ELISA, DNA/RNA probes or impedance. The capture procedure requires approximately 30 min. to perform and reduces the screening time for *Salmonella* by approximately 24 h.

Immuno-magnetic separation procedures have also been developed for other pathogens of concern. Dynabeads® with anti-*E. coli* O157:H7 polyclonal antibodies on their surface can be used for rapid separation of the organism from food samples. Another immuno-magnetic separation procedure for capturing *Listeria* is produced by Vicam (Somerville, MA, USA) and is called Listertest™ Lift. This is used to estimate total viable counts of *Listeria*. The organism is captured by using metal beads coated with anti-*Listeria* antibodies. The beads are then spread onto a Listertest™ Plate and incubated, as appropriate. Colonies are lifted onto a Listertest™ Membrane and an immuno-detection procedure is used to make the *Listeria* visible so that the organisms can be counted. This procedure requires approximately 24 h to complete.

Another means of conducting immuno-magnetic separation of bacteria from samples is called the Matrix Pathatrix method. The Matrix system involves continuous circulation of the entire homogenate or rinse sample over a capture area that enhances vortex-mixing of the sample. During circulation, the sample is incubated at the optimum temperature of the target organism to promote growth. This method was shown to be more effective than the currently-used USDA method and other immuno-capture methods for detecting the presence of *Salmonella* in a variety of food samples. The three types of bacteria that can be detected by the method include *Salmonella, Listeria* and *E. coli* O157:H7.

Mansfield and Forsythe (1993) evaluated the immuno-magnetic-separation technique as an alternative to enrichment broths for detection of *Salmonella*. These authors reported that immuno-separation gave similar numbers of true positives in much shorter time periods than standard enrichment procedures. Only immuno-separation isolated *Salmonella* from spiked garlic granules, demonstrating the possible recovery of sub-lethally injured cells.

Magnetic-separation procedures have gained much attention recently, because they significantly reduce the time required for pathogen screening tests by eliminating selective enrichment. These methods are sensitive, rapid, technically simple and complement DNA probe or ELISA methods.

Polyclonal Enzyme Immunoassay (EIA)

Biocontrol Systems Inc. (Bothell, WA, USA) have developed a polyclonal enzyme immunoassay (EIA) called Assurance® EIA for detecting the presence

of *Salmonella* and *Listeria* in foods. Samples are pre-enriched for 18–24 h and then placed in wells coated with Assurance anti-*Salmonella* or anti-*Listeria* polyclonal antibodies. These polyclonal antibodies selectively capture the antigens on the target bacteria. Assurance EIA uses a blend of antibodies to screen-out common cross-reactors. Most ELISA tests use only one or two broad-spectrum monoclonal antibodies. By using a mixture of specific antibodies, EIA is more able to capture only the desired bacterial species. ASSUR-LINK reagents are added to the wells and the wells are incubated before being washed. The ASSUR-LINK antibodies become linked to the captured antigens of the target bacteria. This step increases the sensitivity of the assay by ensuring a linkage between small bacterial antigens of the target species and the large, bulky conjugate, thus increasing the efficiency of chemical coupling. Such a step also reduces the occurrence of missed couplings, due to stearic hindrance. Conjugate is then added to the wells, which are incubated and washed for a second time. The conjugate binds to ASSUR-LINK and carries an enzyme needed for the color reaction. Finally, a substrate is added. The substrate is cleaved by the enzyme and a yellow color forms in the well. Results can be obtained in as little as 2.5 h following pre-incubation.

Feldsine *et al.* (1992, 1993a) evaluated the polyclonal enzyme immunoassay method for detecting *Salmonella* in a variety of foods and reported that the method is more sensitive than traditional immunoassays, due to the use of polyclonal antibodies. For more than 1000 food samples, including non-fat dry milk, dry egg, black pepper, soy flour, chocolate and ground poultry, there was a 97.2% and a 96.5% agreement respectively between the EIA method and the AOAC/BAM cultural method. The proportion of false-negative results was comparable for all foods except ground poultry. For ground poultry, the EIA method detected significantly more confirmed, positive samples than did the AOAC/BAM procedure (Feldsine *et al.*, 1992, 1993a).

This method is sensitive and can be conducted in less than 2 h, following pre-enrichment. However, it is more labor-intensive than automated methods, such as TECRA® OPUS®.

Enzyme-linked immuno-fluorescent assay (Vidas®)

The Vidas® system (bioMérieux Vitek Inc., Hazelwood, MO, USA) has been adapted from ELISA technology and can be used to detect specific pathogens or identify bacterial isolates in 45 min. A solid phase receptacle (SPR) is a pipette-tip-like disposable device that is coated on the inner surface with antibody used to capture the target antigen. The SPR also acts as a pipettor for accurate sampling and transfer of reagents during the assay. The Vidas® system can be used to screen food samples for the presence of *Salmonella, Listeria* and *E. coli* O157:H7, using different SPRs coated with specific antibodies. To conduct an assay, the appropriate SPR and a reagent strip, containing diluents, antibodies, washes, conjugates and substrate, are inserted in the instrument. The instrument then dips the SPR in the sample well, pre-wash solution, washing buffer (three times), conjugate, washing buffer (three times) and substrate, respectively.

During this automatic procedure, the target bacterium is captured by the antibodies on the SPR surface. Conjugate antibodies containing the enzyme alkaline phosphatase then bind to the bacterium. When the SPR is finally dipped in the well containing the substrate, the MUP couples with the alkaline phosphatase to form 4-methyl-umbelliferone, which is fluorescent. The intensity of the fluorescence is determined with an internal optical scanner. The results are analyzed and a print-out is generated.

Mozola *et al.* (1993) compared an automated immunoassay test system (Vidas®) with an ELISA-based test kit (Listeria-Tek™) for the detection of *Listeria* and found that, of 437 samples of meat, dairy products, vegetables, other foods and environmental swabs analyzed, there was a 93.8% agreement in detection level between the two assays. The Vidas® system produced 46 confirmed positive results and the Listeria-Tek™ detected 42 confirmed positives. False-positive rates of 1.5% and 5.9% were calculated for the Vidas and Listeria-Tek™, respectively (Mozola *et al.*, 1993). Overall, the Vidas® is fully automated, easy to use and accurate. Results can be obtained rapidly; however, the initial cost is high.

20.4 Enumeration methods

20.4.1 Standard plate counts

Standard plate-count methods for enumerating microorganisms from food samples are based on the ability of the organisms to multiply in culture media containing agar to form colonies. It is assumed that each colony-forming unit (cfu) represents a single bacterium that has grown in, or on, the medium in question, at a given temperature and in an appropriate atmosphere, to a point where the mass of cells produced can be observed by eye, so that colonies can be counted.

Approximately 48 h are required for a single bacterial cell to multiply and produce a visible colony on an agar medium (Swanson *et al.*, 1992). Current food-transportation and marketing practices have made it possible for products to be sold and even consumed before traditional plate counts can be obtained and interpreted. However, other means of enumerating bacteria have been developed that significantly reduce the time required to obtain results.

20.4.2 Radiometry

Radiometry is a technique that involves adding radiolabeled ^{14}C to a microbiological culture medium (Rowley *et al.*, 1979). Samples containing the bacteria to be enumerated are then inoculated into the growth medium. As the bacteria multiply, the radiolabeled ^{14}C is incorporated in $^{14}CO_2$, which is then liberated and can be measured. Using selective ingredients in the growth medium, single bacterial species or groups of bacteria, such as coliforms, can be enumerated by means of radiometry (Rowley *et al.*, 1979).

20.4.3 Calorimetry

LKB 2277 BioActivity Monitor

During microbial growth, various metabolic products may be measured as a means of enumerating microbial populations rapidly. Microbial growth results in heat production. The minute amount of heat produced by the metabolic processes of microbes growing in culture, or in any contaminated material, may be measured with the use of extremely sensitive calorimeters (Monk and Wadsö, 1968). The LKB 2277 BioActivity Monitor (LKB-Produkter AB, Bromma, Sweden) is an example of a calorimeter that has been used to estimate microbial numbers (Gram and Søgaard, 1985). The number of bacteria required to produce a sufficient amount of heat to be detected is approximately 10^4 cfu per ml (Lampi *et al.*, 1974) to 5×10^5 cfu per ml (Berridge *et al.*, 1974). Microcalorimetry has been used to estimate microbial numbers in urine (Beezer *et al.*, 1978), canned foods (Sacks and Menefee, 1972), milk (Cliffe *et al.*, 1973) and ground meat (Gram and Søgaard, 1985).

20.4.4 Impedance, conductance and capacitance

Impedance and conductance methods have been used effectively for rapid screening of foods, such as milk (Olivera and Parmelee, 1976; Firstenberg-Eden and Tricarico, 1983; Firstenberg-Eden and Eden, 1984), frozen vegetables, (Hadley *et al.*, 1977), fish (Gibson *et al.*, 1984), beef, ground beef and raw-meat sausage (Firstenberg-Eden, 1983), to determine whether they meet a desired microbiological criterion.

Impedance is defined as the opposition to flow of an alternating electrical current in a conducting material. Conversely, conductance is the ability of an alternating electrical current to flow through a conducting material. As bacteria multiply, they convert large molecules into smaller, more mobile metabolites that change the impedance of the medium. These metabolites increase the conductance and decrease the impedance of the medium. When microbial populations reach a level of 10^6–10^7 cells per ml, a change in the impedance of the medium is observed. Firstenberg-Eden (1985) stated that the time required for this exponential change to occur is known as the impedance detection time (DT).

DTs can be obtained in shorter periods of time than APC (aerobic plate count). Often, results can be obtained in 12 h or less. However, there are several fundamental differences between impedance techniques and APC (Firstenberg-Eden, 1985). Using APC, all bacteria that are able to reach a visible biomass are counted (Firstenberg-Eden, 1983), whereas the impedance technique relies on the measurement of metabolic changes (Firstenberg-Eden, 1985). Because impedance measurements depend on the metabolic changes produced by the fastest-growing bacterium or group of bacteria in the sample, factors, such as medium, time and temperature, become critical. Specific bacteria use different metabolic pathways, depending on the medium in which they are grown. Some end-products of metabolism produce stronger impedance signals than others, when bacteria are allowed to multiply and utilize different substrates in the

medium (Firstenberg-Eden, 1985). Therefore, the substrates that bacteria utilize will determine the by-products they produce and hence are an important consideration in relation to impedance assays.

To estimate APC using impedance, it is essential to use a medium, temperature and gaseous environment that minimizes the differences in generation time for the species in the sample (Firstenberg-Eden and Eden, 1984). By minimizing such differences, the organisms, although different, will multiply at a similar rate and will reach the detection threshold of 10^6–10^7 viable cells at similar times. Using this method, correlations between impedance and plate counts will be highest. By relating DT to APC, future APC can be estimated from impedance readings.

A major advantage in impedance microbiology is that media, temperature and environmental conditions may be manipulated to selectively enumerate only one bacterial species or group of bacteria. For example, with a sample containing 100,000 pseudomonads and 1 coryneform organism, and incubated at 30 ºC, the coryneform would be the first organism to reach the detection threshold level (Firstenberg-Eden and Eden, 1984). At 30 ºC, the generation time of a pseudomonad is four times that of a coryneform, but the coryneform can still multiply and reach 10^6 viable cells before the pseudomonads.

Impedance has been used as a means of enumerating coliforms (Martins and Selby, 1980, Firstenberg-Eden and Klein, 1983, Firstenberg-Eden *et al.*, 1984; Strauss *et al.*, 1984; Tenpenny *et al.*, 1984), fecal coliforms (Mischak, *et al.*, 1976; Silverman and Munoz, 1978, 1979; Rowley *et al.*, 1979) and Enterobacteriaceae (Cousins and Marlatt, 1989) from samples of wastewater, dairy products and meat. Impedance may also be used to predict the shelf-life of foods, to test the sterility of ultra-high-temperature products, to conduct kinetic measurements on microbial growth and to determine the sensitivity of microbes to antimicrobial compounds.

Bactometer M128

The Bactometer (bioMérieux Vitek Inc., Hazelwood, MO, USA) utilizes a disposable module with 16 sterile sample wells. Once samples have been diluted in a culture medium, 1 ml of the mixture is added to each well. The wells are then capped and the module is plugged into an aerobic incubation chamber. Each well has a positive and negative electrode. An electrical current is passed periodically between the two electrodes, and the impedance, conductance and capacitance of the electrical impulse are monitored.

Malthus 2000 Microbiological Analyzer

The Malthus (Malthus Diagnostics, North Ridgeville, OH, USA) utilizes sterile tubes in which samples are placed. The top of each tube, containing the electrodes, is connected to the monitoring system. The tube is then placed in a water bath to incubate. An electrical current is passed periodically between the electrodes, and the conductance of the electrical impulse between the two electrodes is monitored.

RABIT

More recently, a different means of enumerating microorganisms, using impedance measurement, has been developed called the RABIT (Microbiology International, Frederick, MD, USA). Samples are placed in re-usable modules containing a bacteriological culture medium. The module is then placed in an incubation block. As before, an electrical current is passed periodically between the electrodes and the impedance of the electrical impulse between the two electrodes is monitored.

20.4.5 Optical methods

BioSys-128®

A new optical method, called the BioSys-128® (MicroSys Inc., Ann Arbor, MI, USA), has been developed to detect the metabolic changes associated with microbial growth. As organisms multiply and metabolize nutrients in a liquid medium, they change the chemical characteristics of the medium. These chemical changes can be detected using optically-sensitive reagents that change their spectral characteristics as metabolism progresses. Like impedance, when the population of organisms in the sample reaches a 'threshold level', the instrument is then able to detect the spectral change that has occurred and record a detection time (DT). As with impedance, the DT is inversely proportional to the number of organisms in the sample. The higher the concentration of organisms in the original sample, the more rapidly the DT will be reached. This relationship has important practical applications. Samples with high concentrations of organisms will be detected more rapidly and the test result obtained sooner, thereby providing an early warning of highly-contaminated materials. Also, as with impedance, the instrument operates in the range of $1–10^7$ organisms per ml, thereby eliminating the need for any sample dilution. The sensitivity of the assay is one microbial cell, which makes it an excellent means of screening supposedly sterile products for contamination.

To use the system, samples are placed in a vial containing a microbiological culture medium and a color indicator. At the base of the vial is a small compartment containing a semi-solid material. The color indicator in the medium is allowed to diffuse into the semi-solid material. The vial is then placed in the instrument for incubation and monitoring. Samples may be incubated at either high or low temperatures. As organisms multiply in the medium, they produce metabolites that change the color indicator in the semi-solid base. Changes in color are detected with an optical sensor and expressed as optical units.

The dynamic response-curves produced, as the instrument records optical variations in the medium during microbial growth, are very similar to classical microbial growth curves during the logarithmic phase. The curves can be used to study the growth kinetics of cultures and their responses to nutrients, temperatures, preservatives and antibiotics. The curves are slightly different from those produced with the use of impedance techniques in that, since an

electrical current is not involved, there is no electronic drift associated with the curves, while the organisms present adjust to the incubation temperature.

Omnispec™ 4000 Bioactivity Monitor System

Tristimulus reflectance colorimetry is a rapid method for monitoring microbial activity by measuring pigmentation changes that occur in a dye, as the organisms multiply. Any dye used in this assay must show a color change, as the by-products of metabolism shift the pH, oxidation/reduction potential or concentrations of free amino groups in the medium (Manninen and Fung, 1992). Microbial by-products change the color of the dye and a colorimeter, such as the Omnispec™ 4000 Bioactivity Monitor System (Wescor Inc., Logan, UT, USA), is used to measure the total visible spectrum during incubation of samples. Signals from the color-detection system are processed, transferred to the Omnispec computer, converted to L*a*b* color-notation values and stored in the test data file (Manninen and Fung, 1992). As the color changes (e.g. from black to white (L*), green to red (a*), or blue to yellow (b*)), the total number of organisms in the sample initially can be estimated according to the time required for the color of the sample to reach a user-programmed end-point. Manninen and Fung (1992) reported that, once the color detection time is reached, the program calculates either log or antilog values for each sample, using the slope and y intercept values, e. g. [final value = y intercept + (slope $\times$ time interval)].

In general, results can be obtained in less than 15 h with reflectance colorimetry. This assay has been used to estimate microbial populations in minced beef samples (Manninen and Fung, 1992).

20.4.6 Alternative methods for estimating specific microbial populations

Temperature-independent pectin gel (TIPG) method

The TIPG method (3M, St Paul, MN, USA) has been adapted recently to enumerate coliforms. The pectin-based, violet-red bile (VRB) method was found to permit greater recovery of coliforms, with better precision, than the conventional, VRB Agar method (Roth and Bontrager, 1989). Another method that utilizes pectin gel has been developed to enumerate coliforms and *E. coli* on the same plate. Two compounds are used in the ColiChrome 2™ test (3M, St Paul, MN, USA) to detect the presence of the enzymes, glucuronidase and galacturonidase. Glucuronidase is produced by *E. coli*, but not by other coliforms. The presence of this enzyme is indicated by the production of a blue color. Galacturonidase is generally produced by coliforms and the presence of this enzyme is indicated by a red-magenta color. The blue and red-magenta colonies can then be counted separately to enumerate the populations of *E. coli* and coliforms, respectively. Ingham and Moody (1990) reported no significant differences between the Redigel®, Petrifilm™ or standard methods for enumeration of *E. coli*. The advantages in using the TIPG method for enumerating coliforms are similar to those listed for APC, using the same basic method.

Dry, rehydratable-medium films

A variety of dry-medium films have been developed by 3M for the enumeration of specific organisms. These films are similar to those listed below, except that selective media are used in the basal film. Petrifilm™ Coliform Count Plates (3M, St. Paul, MN, USA) have a VRB Agar coating on the basal film. Bacteria that multiply on Coliform Count Plates produce a dark-red spot. Colonies that produce such a spot and form a small gas bubble are counted as coliforms. Nelson *et al.* (1984) and McAllister *et al.* (1988) reported that, in comparison with standard methods for enumerating coliforms, the Coliform Count Plate technique showed a good correlation ($R^2 = 0.93$). Petrifilm™ was described as a practical and accurate alternative for monitoring coliform levels on poultry products from processing facilities (Bailey and Cox, 1987; McAllister *et al.*, 1988). The 3M company have also developed a High-Sensitivity Coliform Count Plate. This plate is larger than other petrifilm plates in order to accommodate a sample of up to 1 g. The sensitivity of the technique is improved, because a single coliform would be detected in a 1 g sample.

Also, 3M has added an indicator dye to the Petrifilm™ Coliform Count Plate and this reacts with glucuronidase produced by *E. coli* to form a blue color. This test is termed the E. coli Count Plate. Using these plates, coliforms and *E. coli* can be enumerated simultaneously. Coliforms on the plate appear as red colonies, with gas bubbles surrounding them, and *E. coli* appears as a blue colony with gas. Results obtained using E. coli Count Plates correlate well ($R^2 = 0.81$) with those obtained using VRB Agar (Matner *et al.*, 1990).

This procedure has also been adapted for the detection of *E. coli* O157:H7. After incubation, the blue, antigen-containing colonies on E. coli Count Plates are transferred to a reactive disc (Okrend *et al.*, 1990). The antigens are then used to capture enzyme-labeled anti-O157 antibodies in the first development step. The location of the antibody is detected in the second step, when the enzyme that is bound to the antigen converts a specific substrate to form a permanent black spot on the disc. Each black spot indicates an *E. coli* O157:H7, presumptive-positive colony. This screening method identifies both negative and presumptive-positive samples within 26–28 h.

Dry-medium films have also been developed to enumerate yeasts and molds (Petrifilm™ Yeast and Mold Count Plate). In addition, a technique is now available to enumerate lactic acid bacteria by first diluting the sample in Mann, Rososa and Sharpe (MRS) broth, then placing the sample onto Petrifilm™ Aerobic Count Plates and incubating the plates under anaerobic conditions. These techniques are similar to the Aerobic Count Plates with regard to their advantages over conventional methods.

20.5 Microbial identification

20.5.1 Miniaturized techniques for identifying bacteria

Fung (1969) developed an inexpensive, miniaturized method for identifying bacterial isolates. A 96-well microtiter plate serves as the master plate.

Microbiological growth-media containing specific biochemical reagents are placed in the wells. The isolate to be identified is first picked from the surface of an agar plate. The isolate is mixed with sterile saline or peptone water and transferred to a sterile receptacle. An inoculating device is used and this consists of 96 stainless steel pins that have been pushed halfway through a piece of balsa wood in precisely the same pattern as the wells of the microtiter plate. After the pins have been dipped in the inoculum, they are placed directly over the microtiter plate containing the different reagents and lowered into them. Following incubation, the wells are examined and the reactions recorded. A chart showing typical biochemical reactions can then be used to determine the identity of the bacterium. This procedure is very similar to biochemical testing using test-tubes; however, it requires much smaller amounts of the media and many tests can be carried out from a single inoculation step, saving space, time and effort.

API®

Various biochemical kits have been developed for the rapid identification of certain groups of organisms. API® (bioMérieux Vitek Inc., Hazelwood, MO, USA) have kits that are designed specifically to identify the following groups of organisms: Enterobacteriaceae (API 20E®), Gram-negative, non-Enterobacteriaceae (API NFT), *Campylobacter* spp. (API CAMPY), staphylococci and micrococci (API Staph-IDENT®), streptococci (Rapid STREP), corynebacteria (Rapid CORYNE), *Listeria* (API LISTERIA), anaerobes (API 20A), *Lactobacillus* spp. (API 50 CH) and yeasts (API 20C). To use these kits, the test organism is suspended in saline and a strip containing various substrates for biochemical assimilation tests is inoculated with the suspension. The strip is incubated for a specified period of time at a specific temperature. After incubation, the biochemical reactions are determined, either manually or automatically. Some biochemical reactions require additional reagents to facilitate interpretation. The set of biochemical reactions obtained corresponds to an allotted number. By comparing the number to those in a database, the organism is identified.

Gooch and Hill (1982) reported that the API 20E® system identified organisms to both genus and species on 90.2% of occasions, when compared with results obtained by conventional methods. Cox *et al.* (1984) found that the advantages associated with the API 20E® system, as opposed to other rapid kits, are as follows: results are easy to read, there is an extensive database and the tests are relatively rapid and easy to set up. The disadvantages are that test units are difficult to stack in the incubator, cumbersome to handle and reactions are not always easy to interpret.

Micro-ID™

The Micro-ID™ System (Remel Inc., Lenexa, KS, USA) is a rapid method for identifying Enterobacteriaceae. Each test-strip contains twenty filter-paper discs that are impregnated with various reagents. An inoculum of the test bacterium is

placed in each of a series of wells containing one of these discs. The reagents on the discs include a biochemical substrate that reacts with a particular microbial enzyme and a detection system that reacts with the metabolic end-product of this process to yield an easily-identifiable color change within 4 h of incubation. The series of color changes is used to obtain a number that is compared to those in a database to identify the bacterium.

Gooch and Hill (1982) observed that the Micro-ID™ System identified organisms to both genus and species on 93.5% of occasions, when compared with results obtained by conventional methods. Cox *et al.* (1984) reported that, when compared with other rapid methods, the Micro-ID™ strip was easier to inoculate, had a shorter incubation time, required little time for the addition of reagents and was fairly easy to read. The disadvantage was that the reactions were not always easy to interpret.

IDS RapID Systems

To identify microbes using IDS RapID Systems (Remel, Lenexa, KS, USA), a colony is mixed with an inoculation fluid to give an appropriate cell density. This suspension is then used to inoculate a test strip containing 10–18 biochemical tests, depending on the type of bacterium or yeast to be identified. Upon addition of the inoculum, the dry reagents in the test wells are re-hydrated. As the organism utilizes the biochemical reagents present, color changes occur. The resulting pattern of positive and negative tests is compared to those in a database to identify the isolate. Strips are available to identify Enterobacteriaceae, non-fermentors, anaerobes, streptococci, *Leuconostoc*, pediococci, *Listeria, Neisseria, Haemophilus*, urinary-tract bacteria and yeasts.

Celig and Schreckenberger (1991) determined the efficacy of the RapID ANA II for identifying anaerobes. When identification to genus level only was determined, 96% of anaerobic, Gram-negative bacilli, 94% of *Clostridium* spp., 83% of anaerobic, non-sporing, Gram-positive bacilli and 97% of anaerobic cocci were identified correctly. When identification to both genus and species was examined, 86% of anaerobic, Gram-negative bacilli, 76% of *Clostridium* spp., 81% of anaerobic, non-sporing, Gram-positive bacilli and 97% of the anaerobic cocci were identified correctly.

Biolog

The Biolog System (Biolog Inc., Hayward, CA, USA) utilizes 96-well microtiter plates that are pre-loaded with metabolic test substrates and reagents for identifying isolates. Each microtiter well contains a different carbon source and Biolog's dry redox reagents. The chemicals are re-hydrated by inoculating with a microbial cell-suspension of specific turbidity. During incubation, the organism utilizes carbon sources in some of the wells. If a carbon source is used by the bacterium, a redox reaction takes place that changes the color of the well contents from clear to purple within 4–24 h. The color change may be determined manually or by using a microplate reader in conjunction with a computer. Once the plates have been read, the MicroLog™ software relates the

information to a number that is compared with those in a database for identification. The system may be used to identify many species of Gram-positive and Gram-negative bacteria. A system has also been developed specifically for the identification of *Escherichia* and *Salmonella* spp.

Biolog has developed MT MicroPlates™ that are 96-well microtiter plates containing the same nutrient base and color reagents as the GN Microplate™, but without added carbon sources. These plates may be tailored for specific identification purposes by adding selected carbon sources. Using this technique, the metabolic capabilities of certain microbial isolates may be studied.

Klingler *et al.* (1992) evaluated the Biolog System for its ability to differentiate between various microbial isolates. Of the 39 isolates studied from the American Type Culture Collection, the Biolog System accurately identified 98% to genus level and 76% to species level within 4–24 h. Overall, the Biolog System is easy to use, fairly inexpensive and capable of identifying many different organisms; however, proper inoculation of the microtiter plate may require practice to obtain consistent results. During the manipulations involved, the technician conducting the test must be careful not to transfer any nutrients to the microtiter wells. If nutrient medium reaches a microtiter well, it will support microbial growth and cause a redox reaction, even though the bacterium is unable to utilize the carbon source in question. In such cases, the bacterium is either incorrectly identified or not identified at all.

BBL® Crystal™

The BBL® Crystal™ system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) is a relatively new method for identifying microbial isolates. There are two Crystal™ test kits available: the Rapid Stool/Enteric ID Kit (RS/E kit) and the Enteric/Nonfermenter ID Kit (E/NF kit). Each of these kits is based on modified conventional and chromogenic substrates (30 in total) contained within a novel plate (Holmes *et al.*, 1994). Each kit comprises a plastic base containing reaction wells to which, following inoculation, is clipped a lid with dehydrated substrates on the tips of plastic prongs. After 3 h incubation for the RS/E kit and 18–20 h for the E/NF kit, the results are interpreted visually and recorded manually. The data are then converted to a 10-digit profile number that is compared to those in a database for identification purposes (Holmes *et al.*, 1994).

When compared to API® and Vitek® (bioMérieux Vitek Inc., Hazelwood, MO, USA), the Crystal™ system performed well. Robinson *et al.* (1995) reported that, of 512 Gram-negative bacilli (381 Enterobacteriaceae and 131 non-enteric bacilli), the Crystal™ system correctly identified 95.5% to genus and species level. The system correctly identified 93.9% of the isolates within 24 h, without any supplementary testing. The authors also found that identification errors associated with this system were not only infrequent, but appeared to be randomly distributed among the genera evaluated.

Holmes *et al.* (1994) evaluated Crystal RS/E and E/NF to determine how effectively they could identify Gram-negative isolates. In all, 203 and 266 strains

were analyzed using the RS/E and E/NF kits, respectively. The RS/E and E/NF kits identified correctly 91% and 93% respectively of the Enterobacteriaceae tested. The authors reported that the systems were easy and safe to use.

Overall, when compared to conventional methods, these miniaturized biochemical techniques for microbial identification are accurate, require less incubator space, need no time, labor or materials for preparation and results can be obtained much more rapidly. Also, the quality of the materials used in the kits is very good. Errors associated with sterilization failures or poor preparation of media are avoided. However, the kits are subject to inoculation error, because a specified concentration of microbial cells must be used in the test suspension or the results will be inaccurate. Also, microbial cultures that cannot be emulsified in saline are difficult to deal with and, in some cases, are impossible to identify with these miniaturized systems.

Optical density method

The Vitek® AutoMicrobic System (AMS, bioMérieux Vitek Inc., Hazelwood, MO, USA) is one of the most versatile of the rapid methods available for microbial identification. To identify an isolate, a colony is mixed with a 0.45% saline solution in a test-tube to give a specific turbidity, depending on the type of organism to be identified. The microbial suspension is used to inoculate a transparent card. One end of a small plastic tube is inserted in the test card and the other end is placed in the suspension in the test-tube. The card and tube with the suspension are placed in a stand. The stand is transferred to a filler that uses a vacuum to pull the suspension up into each of 30 test wells containing biochemical reagents. The card is sealed, inserted in a holder and placed in the AMS. As bacteria utilize the reagents in the card, the optical density for each test well changes. This is measured hourly. The series of readings is compared to those of a standardized database to identify the organism. Usually, microbial isolates can be identified within 4–18 h and as many as 120 samples may be analyzed simultaneously.

Eight different test cards are available, depending on the microbe to be identified, including the following: Gram-negative (GNI), Gram-positive (GPI), Nonfermenter (NFC), Bacillus (BAC), Anaerobe (ANI), Yeast (YBC), Bioburden Enumeration (BIO) and Assay cards (ASC). The BIO card is used to enumerate microbial populations in liquid samples. The ASC card is used to measure the strength or efficacy of antibiotics, vitamins, biocides or preservatives by monitoring the multiplication of organisms exposed to these compounds.

Bailey *et al.* (1985) evaluated the AMS to determine its ability to identify stock cultures and freshly-collected Enterobacteriaceae from ground beef, processed chickens, frozen pot pies and commercial poultry feed. The AMS correctly identified 135 out of 136 (99.3%) of the stock cultures and 160 out of 163 (98.2%) of the fresh isolates to species level. Robinson *et al.* (1995) compared the Crystal™, API® 20E and Vitek®, and reported that of 381 Enterobacteriaceae, the AMS identified 96.1% correctly.

Twelve laboratories evaluated the GNI card for identification of Enterobacteriaceae and found that the AMS correctly identified 96.7% of

Salmonella strains, 97.0% of *E. coli* and an average of 93.0% of the other genera (Knight *et al.*, 1990). The AMS and the GNI card have been approved for interim, official first-action by the AOAC, as a screening method for the presumptive identification of *Salmonella, E. coli* and Enterobacteriaceae isolated from foods. The Vitek AMS is able to identify many organisms accurately. The database is extensive, many samples can be identified at any one time and the cost per test is low; however, the initial cost for the equipment is high.

20.5.2 Headspace analysis

Gas chromatography (GC) has been shown to be an effective means of characterizing microbes chemically (Larsson and Mardh, 1977). To identify a bacterium using GC, the atmosphere above an actively-growing population is sampled and analyzed to determine the volatile by-products that are evolved, as substrates in the medium are metabolized. Because particular microbial species produce specific metabolic by-products, differences in chromatographic patterns can be used to differentiate between them. Once these volatile by-products have been determined, a database is used to identify the bacterium. Traditionally, this technique has been used for rapid identification of bacteria associated with human infections; recently, however, studies have been conducted in which headspace analysis has been used as a means of characterizing spoilage odors produced by bacteria growing on fresh poultry (Viehweg *et al.*, 1989). This method is expensive and is recommended only for certain applications.

20.5.3 Cellular fatty acid analysis

Bacteria may also be identified by extracting cellular fatty acids, analyzing them by GC and then comparing the chromatographic analyses to a database. For many years, analyses of short-chain or volatile fatty acids (VFA) have been used to identify anaerobic bacteria (Sasser, 1990a). Researchers have used VFA between nine and twenty carbon atoms long to characterize bacterial genera and species, especially for non-fermentative, Gram-negative bacteria. Since the development of fused-silica capillary columns, it has become possible to use GC for methyl esters of whole-cell fatty acids to identify many species of bacteria (Sasser, 1990a).

More than 300 fatty acids and related compounds are found in bacteria. Either the presence or absence of these compounds and, if present, their quantification can be used to separate bacterial species effectively. Gas-liquid chromatography (GLC) of fatty-acid methyl esters (FAME) was shown to be an effective tool for identifying bacteria that are important in clinical and industrial settings (Miller, 1987; Moore *et al.*, 1987; Stockman *et al.*, 1987; Osterhout *et al.*, 1989) and identification by GLC closely parallels results from ribosomal RNA and DNA homology analysis (Sasser and Smith, 1987).

MIDI Sherlock® Microbial Identification Systems (Newark, DE, USA) have developed databases for FAME profiles to identify aerobic and anaerobic bacteria and yeasts. The MIDI system comes with a Hewlett-Packard capillary gas-

chromatograph, autosampler, detector, computer, printer, Microbial Identification Software (MIS) and the Library Generation Software (LGS) package. The LGS contains two cluster-analysis packages that have 'tracking' capabilities. The Dendrogram and 2-D Plot programs use data obtained from fatty-acid analyses of microbes and print out easy-to-understand plots of the microbial isolate in question and an indication of its relationship to other organisms (Sasser, 1990b).

These systems are excellent for differentiating between different organisms; however, the databases need further development and the initial cost for the equipment is high.

20.5.4 Riboprinting

A new, fully-automated method called the RiboPrinter™ Microbial Characterization System (DuPont, Wilmington, DE, USA) has been developed and is based on obtaining the DNA 'fingerprint' of the organism. The genetic fingerprint (RiboPrint™) pattern is generated from the rRNA operons and other surrounding regions of the bacterial genome. rRNA genes in bacteria are highly conserved; however, they are completely unique. Thus, RiboPrint™ patterns can be used to separate bacterial isolates. To analyze a sample with this system, DNA is extracted from bacterial cells and is fragmented by means of a restriction enzyme. DNA fragments are separated by their molecular weight, using electrophoresis and transferred to a membrane. The DNA fragments on the membrane are hybridized with a DNA probe and a chemi-luminescent label is introduced. Light is emitted from the hybridized fragments and an image of the pattern generated is captured with a camera. A computer analyzes the RiboPrint image and compares it to a database to identify the bacterium in question. A distinct advantage in using this type of system is that bacterial isolates can be characterized below the species level. More than 25 different RiboPrint™ patterns have been observed for *L. monocytogenes* alone. This makes the system extremely valuable for tracing bacterial isolates implicated in cases of foodborne illness. For example, RiboPrint™ patterns obtained from a particular bacterium isolated from a stool sample could be compared with patterns from isolates obtained at a suspect food-service establishment. Likewise, this system can be used to exonerate a company that may have been implicated wrongly in an outbreak of foodborne illness. The RiboPrinter™ Microbial Characterization System is fully automated; however, the initial cost of the system and the cost of analyzing each sample are very high.

20.6 Monitoring microbial growth: kinetic optical density

Bioscreen

Bioscreen (Labsystems OY, Helsinki, Finland) is a microbiological robot-analyzer that can be used to monitor the growth of most microorganisms in almost any culture medium. The Bioscreen System can perform up to 200 analyses simultaneously, using multi-well plates. A microprocessor controls the

dispensing, incubating/shaking and kinetic optical-density (OD) measurements under sterile conditions. As bacterial populations multiply in the sample, the kinetic OD is monitored and the computer software converts this data into a variety of report formats, including growth curves, colony forming units, toxicity values and bioassay results.

20.7 Selecting the right technique

When deciding on the rapid method that is most appropriate for a particular company, the considerations discussed below are relevant.

20.7.1 Time needed to obtain results

How much is it worth for each 24 h saving in the time needed to obtain results? If the product is extremely perishable and never frozen, time is money and rapid methods are essential. If the product is of high value, such as poultry breast meat in the USA, then the need for a same-day test result increases. If the product is value-added, such as fully-cooked breast fillets, and will be eaten directly from the package without further cooking by a person susceptible to food-borne illness, it is extremely important to have the analytical results before releasing the product for sale. Thus, for this type of product, same-day results are almost a necessity and the added cost of using a same-day assay is well worthwhile. On the other hand, if the product is to be frozen and is not so perishable, the company has much more time to gather results and make decisions. In this case, it is much less likely that the company would have to be concerned about people consuming the food prior to the test results becoming available. Also, in the event that the company is testing products in an effort to meet governmental regulations, but never detects the bacterium in question, then time is not nearly as important and a longer, less-expensive test should be considered. An example of a very high-speed detection method for *E. coli* O157:H7 would be to couple the Matrix immuno-magnetic separation procedure (*c.* 5 h) with PCR (*c.* 2 h) to provide a total testing time, from receiving the sample to confirmation, of 7 h. In this way, the product can be tested and released within a processing shift.

20.7.2 Cost

If a company tests many samples per day, then cost can become a major issue. For example, a company in the USA tests approximately 80 samples per day for *Salmonella, Listeria* and *Staphylococcus aureus*. Because this company is not concerned with obtaining results on the same day or even the next day, they have chosen to use very economical test kits. An example of such methods to test samples for pathogens would be to use conventional pre-enrichment and selection procedures involving broth cultures and then employ an immuno-precipitate method, such as the Neogen Reveal or BioControl VIP test strips.

20.7.3 Skill level and availability of labor

Poultry companies must match their rapid tests to the skill level of their employees. If employees skilled in microbiological techniques are not available, or the company cannot afford someone of this calibre, then methods that require very little knowledge of microbiology should be chosen. For example, in a small company that has a technician working in the laboratory with no real microbiological training, it would not be appropriate to use immuno-magnetic separation coupled with PCR. A much more skilled individual would be needed for this type of assay. Instead, a very simple procedure, requiring few transfers, very little preparation of media and almost no interpretation of results would be preferable. The Vidas fluorescent immunoassay would be an excellent choice under these conditions, because it is fully automated. In fact, many poultry companies that have unskilled labor have chosen this method.

In developing countries, where labor is not expensive, much more work can be undertaken by the workforce. For example, methods requiring considerable media preparation, many transfers and manual plate-counting would not be precluded, because the labor is available to meet these requirements. On the other hand, in countries where technicians are highly paid and very hard to find, methods requiring few steps and little manual preparation would be preferable. Overall, selection of the right method for a particular company involves balancing the necessity for speedy results, the price that the company is willing to pay for the test and the skill level and expense associated with the labor that is available to the company. After taking these factors into account, it is much easier to decide between the numerous methods that are available.

20.8 Future trends

The ideal rapid method for the detection and enumeration of microorganisms in foods would be a ‘ray gun’ device that is pointed at the product and gives a reading. Because this method does not yet exist, scientists are trying to develop ways of reducing the time and expense associated with conducting microbiological assays. One such method involves the use of biosensors. These are usually constructed of antibodies against the target organism that are attached to a disc, glass slide or tube. A laser is passed over the surface of the disc, slide or tube interior and an initial reading is taken. Then, the sample is passed over or through the biosensor and any target organisms that are present will immediately attach to the antibodies on the surface of the biosensor. When this occurs, the laser is then deflected and a detector registers the deflection. This occurs immediately, in real-time, and detection has been achieved. However, these assays are very difficult, if not impossible, to use with samples that contain high levels of fat, such as poultry carcass rinses or ground beef samples. To date, no really effective sampling method has been developed to make biosensors a reality for the industry.

Other scientists have been using bacteriophage to detect bacteria. The idea is

that if a certain virus (one that is specific for the target organism) is used to infect the organism, the virus will be able to insert a lux gene into the bacterium to make it produce light. The bacterium may be detected immediately by measuring the light produced at the surface of the product, using a specialized light meter.

Efforts have also been directed at developing an extremely sensitive chemical detector. The idea here is that *Salmonella* and *Listeria* each produce very specific metabolic by-products. If a sensor is passed over the top of a poultry product and these by-products are detected, then the bacterium can be assumed to be present on the product. This is another real-time method for detecting pathogenic bacteria. However, these methods suffer from two major problems. Usually, the chemical (metabolic by-product) to be detected is only there in parts per billion or perhaps, at most, parts per million. In addition, the background readings (initial readings) usually vary and fluctuate more than parts per million, so that it is impossible to know whether the fluctuation in the sensor readings is due to the detection of a metabolic by-product or the background is merely fluctuating wildly. Despite the difficulties, the search for the ideal rapid method is bound to continue.

20.9 Sources of further information and advice

20.9.1 Texts

Compendium of Methods for the Microbiological Examination of Foods, 4th edn. Edited by Frances Pouch Downes and Keith Ito. American Public Health Association. 800 I St. Northwest, Washington, D.C. 20001. This book is an excellent source of sampling and testing methods. It includes many rapid methods and gives a description of how each test works.

Bacteriological Analytical Manual. US Department of Health and Human Services, US Food and Drug Administration, Center for Food Safety and Applied Nutrition. 5100 Branch Parkway, College Park, MD 20740-3835. This is an important source, especially for sampling. It describes in detail the various sampling methods used for different types of food. For example, it describes how cooked, ready-to-eat products should be handled in comparison with raw products.

20.9.2 Workshops

1. Rapid methods and automation in microbiology. Dr Daniel Y C Fung, Kansas State University, USA. Thousands of participants from countries around the world have attended this workshop, which has been held in June or July each year for the past 23 years. The participants are able to attend presentations by the world's experts in all fields of rapid methods and automation, as well as conduct hands-on testing with many rapid and

automated methods in the setting of a food microbiology laboratory. This is the most comprehensive course of its kind in the world.

2. Current concepts in foodborne pathogens and rapid and automated methods in food microbiology. Dr Pernendu C Vasavada, University of Wisconsin, River Falls, USA. Thousands of participants from around the world have attended this workshop, which is usually held in October each year, for the past 22 years. Participants attend seminars held by the world's experts in rapid and automated methods and are able to see the rapid methods in a display setting, while discussing the technology with a company representative. This is also an excellent course.

20.9.3 Agencies

AOAC International
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Gaithersburg, Maryland 20877-2417, USA
+1-301-924-7077 (worldwide)
or 1-800-379-2622 (toll-free from North America)

ISO (International Organization for Standardization)
1, rue de Varembé, Case postale 56
CH-1211 Geneva 20, Switzerland
+41 22 749 01 11

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21

Modified atmosphere packaging and the safety of poultry meat

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21.1 Introduction

An interest in the microbiology of meats soon followed from the founding of bacteriology as a science in its own right (Davies and Board, 1998). Since poultry is considered a highly perishable food, with a shelf-life that rarely exceeds ten days after processing, even at chill temperatures (Marenzi, 1986), packaging systems have been developed that have the following attributes: (i) protection of the meat from external microbial contamination and (ii) the ability to extend shelf-life by suppressing microbial growth. One such system is modified atmosphere packaging (MAP).

A modified atmosphere is obtained by altering the natural composition of air (78% N_2, 21% O_2, 0.03% CO_2 and traces of noble gases) to provide an alternative gaseous environment for enhancing the storage-life and visual quality of foods (Phillips, 1996). This can be achieved by using active or passive MAP. Active modification involves the displacement of air in the package and its replacement with a desired mixture of gases. On the other hand, passive modification requires the product to be packaged in an appropriate gas-barrier film, so that the desired atmosphere develops naturally as a consequence of residual respiration in the product or microbial metabolism, while there is usually some gas diffusion through the film itself (Moleyar and Narasimham, 1994; Zagory, 1994; Lee *et al.*, 1996). The principal gases used in MAP for preservation of meat and poultry are CO_2, O_2 and N_2. These gases are combined in three ways: inert blanketing, using N_2; semi-reactive blanketing, using CO_2 +

N_2 or $O_2 + CO_2 + N_2$; or fully-reactive blanketing, using CO_2 alone or $CO_2 + O_2$ (Blakistone, 1993; Moleyar and Narasimham, 1994).

It has become clear that MAP can extend product shelf-life with almost any combination of CO_2, N_2 and O_2 (Stanbridge and Davies, 1998). In practice, the extended shelf-life should cover not only the time from production to marketing, but the additional time taken for the product to be purchased and then stored in a household refrigerator until it is consumed. Numerous studies have aimed to evaluate the effects of elevated CO_2 atmospheres on poultry in bulk packages, chicken quarters (Hotchkiss *et al.*, 1985, Baker *et al.*, 1986) and ground poultry meat (Baker *et al.*, 1985), in order to assess the effectiveness of this technology for long-term storage. Moreover, much work has been focused on the behaviour of organisms such as pseudomonads and lactic acid bacteria (Garcia-Lopez *et al.*, 1998; Holzapfel, 1998). However, concern has been expressed by regulatory authorities (Gill, 1988), food industry groups (Anon., 1988) and others that MAP may represent an undue safety hazard. Such concern relates to the potential growth of those pathogenic bacteria that could survive and/or grow under MAP, even at refrigeration temperatures (Silliker and Wolfe, 1980; Palumbo, 1987). Despite this, it will be evident here that only a few studies have examined the effect of MAP on the growth/survival of foodborne pathogens on poultry, particularly the psychrotrophic strains. In addition, this chapter will address the importance of studying microbial interactions in stored poultry and the physico-chemical changes that occur during storage of the meat under MAP conditions. The information thus obtained will lead to an understanding of the mechanisms of spoilage and provide a better means of evaluating and/or predicting the potential risks associated with the technology in question. This will ensure that it is only used when there are real benefits to all concerned.

21.2 Microbial spoilage of poultry

21.2.1 Precepts of the poultry meat ecosystem

In the earliest work on spoilage of poultry muscle, breast and leg, received particular attention (McMeekin, 1975, 1977). Spoilage of poultry meat can be thought of as an ecological phenomenon that encompasses the changes occurring in low molecular weight compounds, such as lactate, glucose and amino acids, during the proliferation of those bacteria that comprise the microbial association of the stored meat. The prevalence of a particular microbial association on poultry muscle depends on factors arising during processing, transportation and storage of the product in the marketplace. It is well established that, in food systems, five categories of ecological determinants, i.e. intrinsic, extrinsic, implicit, processing and emergent effects (Mossel, 1983; Gould, 1992; Odum, 1993) influence the development of the particular (ephemeral) microbial associations. They also determine the rate of attainment of a climax population, the so-called 'ephemeral (specific) spoilage organisms'

(E(S)SO), i.e. those organisms that fill the niche available by adopting particular ecological strategies. The strategies developed by the E(S)SO are the consequence of environmental determinants, such as the application of stress (abiotic factors, i.e. intrinsic, processing, extrinsic), any destructive or enrichment disturbance of the ecosystem, such as a sudden event that provides newly available energy sources for exploitation, and the incidence of competitors for carbon sources, oxygen or other substances, e.g. ferric ions (Boddy and Wimpenny, 1992). For this reason, scientists and technologists involved in poultry meat production should attempt to control factors such as temperature, or modify some or all of the parameters noted above to extend the shelf-life of the product, and to provide poultry products with an acceptable shelf-life. The use of MAP can be regarded as suitable for the purpose.

21.2.2 Ephemeral spoilage organisms

It is well documented that the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, and the temperature and other conditions of storage and distribution are the most important factors that determine the microbiological quality of poultry meat (Davies and Board, 1998). Indeed, as the inherent antimicrobial defence mechanisms of the live animal are destroyed at slaughter, the resultant meat is liable to rapid microbial decay. Some of the microorganisms involved could be derived from the intestinal tract of the animal and others from the environment with which the animal or carcass had contact before or during processing. For example, studies on the origins of contaminants have shown that the source of Enterobacteriaceae on meats is more often the work surfaces in the abattoir rather than direct faecal contamination. Moreover, psychrotrophic bacteria can be recovered from a variety of such surfaces, as well as from carcasses and cut portions at all stages of processing (Bolder, 1998).

As mentioned above, many studies on the microbiology of meat and poultry have established that spoilage is caused by only an ephemeral fraction of the initial microbial association (see review by Nychas *et al.*, 1998). This concept has contributed significantly to our understanding of muscle-food spoilage. The range of microbial genera found on poultry meat is given in Table 21.1. A consortium of bacteria, commonly dominated by *Pseudomonas* spp., is, in most cases, responsible for spoilage of poultry meat stored aerobically at chill temperatures. It is now well established that, under these conditions, species such as *Ps. fragi* and *Ps. fluorescens* are the most important. When the population of pseudomonads reaches an arbitrary level of 10^7–10^8 per cm^2, 'off'-odours and slime formation are apparent. In fact, these characteristics become evident when the pseudomonads have exhausted the glucose and lactate present in meat and begin to metabolise nitrogenous compounds, such as amino acids (Nychas and Tassou, 1997).

Cold-tolerant Enterobacteriaceae (e.g. *Hafnia alvei*, *Citrobacter freundii* and *Enterobacter cloacae*) also occur on chilled meat stored aerobically (Zeitoun *et*

Table 21.1 The genera of bacteria and yeasts most frequently found on poultry

Bacteria	Bacteria	Yeasts
Acinetobacter	*Kluyvera*	*Candida*
Aeromonas	*Kurthia*	*Debaryomyces*
Alcaligenes	*Lactobacillus*	*Trichosporon*
Alteromonas	*Leuconostoc*	
Arthrobacter	*Listeria*	
Bacillus	*Micrococcus*	
Brochothrix	*Moraxella*	
Campylobacter	*Neisseria*	
Carnobacterium	*Pantoea*	
Chromobacterium	*Pediococcus*	
Citrobacter	*Planococcus*	
Clostridium	*Plesiomonas*	
Corynebacterium	*Proteus*	
Enterobacter	*Pseudomonas*	
Enterococcus	*Serratia*	
Escherichia	*Streptococcus*	
Flavobacterium	*Streptomyces*	
Hafnia	*Staphylococcus*	

Source: Stanbridge and Davies (1998).

al., 1994), but they do not contribute significantly to the predominant microbial associations in terms of numbers. The Enterobacteriaceae as a whole are more often regarded as indicators of food safety.

Brochothrix thermosphacta and certain lactic acid bacteria have been detected among the aerobic spoilage flora of chilled poultry. These organisms have been isolated from carcasses during dressing, chilling and boning (Bolder, 1998). Both types of bacteria are among the main causes of spoilage that is recognized as a souring of the meat, rather than putrefaction (Kakouri and Nychas, 1994). This kind of spoilage is commonly associated with poultry meat in MAP and involves competition between facultatively anaerobic, Gram-positive organisms. It contrasts with the other situation that occurs, involving competition between Gram-negative bacteria (pseudomonads and Enterobacteriaceae). The changes occurring in either situation are related to the type, composition and size of the microbial association, and to the types and availability of energy substrates in the meat. Indeed, the kind and extent of spoilage is governed by the availability of low-molecular-weight compounds, e.g. glucose, lactate (Nychas *et al.*, 1998). Ultimately, overt spoilage is due to the catabolism of nitrogenous compounds and amino acids, as well as secondary metabolic reactions. Finally, the deliberate addition of Gram-positive organisms (cultures of lactic acid bacteria) to suppress development of the Gram-negative flora represents a different situation and an approach known as 'biopreservation' (Gombas, 1989; Holzapfel *et al.*, 1995).

21.3 Changes in MAP and their effects on survival and/or growth of pathogens

21.3.1 Microbial changes in MAP

The initial microflora of poultry is similar to that of red meat and consists of mesophilic and psychrotrophic microorganisms (Dainty and Mackey, 1992; Bolder, 1998): see Table 21.1. However, McMeekin (1975, 1977) noted differences in the composition of chicken breast and leg muscle, while different penetration rates for various bacteria were reported by Thomas *et al.* (1987). Storage conditions affect both the types of organism that can grow and their growth-rates. During aerobic storage under chill conditions, pseudomonads tend to dominate, because of their shorter generation times, compared to the other members of the microbial association. They are also able to catabolise a variety of nutrients, especially glucose, and then lactate, gluconate and nitrogenous compounds, such as amino acids, proteins, creatine and creatinine. Changing the gaseous environment and increasing the concentration of CO_2 results in a change in the dominant flora from Gram-negative to Gram-positive, which consists mainly of lactic acid bacteria and *Br. thermosphacta* (Stanbridge and Davies, 1998).

Hotchkiss *et al.* (1985) determined the shelf-life of chicken quarters when stored at 2 ºC under 0–80% CO_2. Elevated CO_2 concentrations resulted in lower aerobic counts and extended shelf-life in terms of organoleptic properties including internal and external colour, odour, flavour, tenderness, juiciness, feel and appearance of the meat. Microbial differences between samples stored aerobically and those in MAP were both qualitative and quantitative. The initial flora comprised *Staphylococcus* spp. (29%), pseudomonads (71%) and *Lactobacillus* spp. (0%). After storage at 2 ºC for 35 days in 80% CO_2 + 20% air, the respective proportions were 0, 1 and 99%. The corresponding figures for aerobically stored samples were 11, 89 and 0%, respectively. Similar results were reported for ground chicken stored in MAP (Baker *et al.*, 1985).

Packaging of chicken breasts or thighs in 100% CO_2 was clearly more effective than vacuum packaging at 3 ºC and 10 ºC in terms of delaying microbial growth and the occurrence of physico-chemical changes in the meat, while the least effective atmospheres were 100% N_2 followed by 20% CO_2 + 80% O_2 (Kakouri and Nychas, 1994). In this study, lactic acid bacteria were the predominant population in packs containing 100% CO_2. Co-dominance of this group with *Br. thermosphacta* was observed in vacuum packs, while *Br. thermosphacta* alone predominated in 100% N_2 and 20% CO_2 + 80% O_2. Growth of pseudomonads was suppressed in all cases. There were no significant differences between breast and leg meat in the spoilage associations that developed. Sawaya *et al.* (1995a) monitored the microbiological, chemical and sensory changes that occurred in chicken carcasses stored under MAP (70% CO_2 + 30%N_2, 30% CO_2 + 70% N_2) at 2, 4, 7 and 9 ºC. They found that the shelf-life in 70% CO_2 + 30% N_2 was approximately three times higher than that of air-stored samples at all four temperatures. Reducing the concentration of CO_2 to

30% resulted in a lower, but still significant extension in shelf-life. Overall, MAP storage delayed growth of all members of the aerobic microbial association. Moreover, MAP suppressed the production of microbial metabolites, especially at lower temperatures. In another study, Jiménez *et al.* (1997) observed that packaging in 70% CO_2 + 30% N_2 and storing at 4 ºC extended the shelf-life of chicken breasts so that they kept for up to 21 days, compared to only five days for aerobically stored samples.

21.3.2 Differences between breast and leg muscle, and physico-chemical changes in MAP poultry

Cassens and Cooper (1971), Foegeding (1987) and Morita *et al.* (1987) have described the biochemical and physiological differences between red (slow) and white (fast) muscle. Leg (which contains at least ten different muscles) and breast (two muscles) represent respectively the red and white muscle in poultry. It is well established that chicken breast contains larger amounts of soluble proteins, glucose and lactic acid, but has less pigment (e.g. myoglobin) than leg (Lea *et al.*, 1969; Cassens and Cooper, 1971; Crespo and Ockerman, 1977a,b; Ledward, 1984; Kakouri and Nychas, 1994; Nychas and Tassou, 1997). These differences may explain why the two kinds of muscle do not spoil in the same way and have a different influence on the growth or survival of pathogenic bacteria, and production of toxins. The extensive studies on chicken muscle (McMeekin, 1975, 1977; Sooltan *et al.*, 1987; Thomas *et al.*, 1987; Nychas and Board, 1991; Schmitt and Schmidt-Lorenz, 1992a,b; Kakouri and Nychas, 1994; Nychas and Tassou, 1997) have shown that:

1. the microflora developing on chicken breast and leg differs
2. the generation times for bacteria growing on lower leg meat were faster than on breast meat
3. various bacteria showed different penetration rates in the two kinds of muscle and
4. enterotoxin production by *Staph. aureus* differed between the two.

The key chemical changes associated with the growth of pseudomonads in meats have been studied extensively, using model systems, e.g. meat juices, gel cassette systems, sterile meat and naturally contaminated meat or poultry (McMeekin, 1975; Gill, 1976; Molin, 1985; Drosinos and Board, 1994; Kakouri and Nychas, 1994; Tsigarida *et al*., 2003). Not only were *Pseudomonas* spp. found to catabolise creatine and creatinine under aerobic conditions, but the phenomenal amount of ammonia released, and the associated increase in pH, were inextricably linked to the catabolism of these substrates. Ammonia can be produced by many different microbes in the course of amino acid metabolism. Other volatiles found in spoiled meat include ethanol, acetone, propan-2-ol, dimethylsulphide, propan-1-ol, ethylcate, 2,3 butandione, acetic acid, diacetyl, hexane, heptane, pentanol, heptadiene, acetoin and octane, 2,3, butanodiol (Nychas *et al.*, 1998).

Storage of poultry muscle in air or MAP affects the concentrations of glucose and L-lactate present. For both substrates, changes in concentration were more marked at 10 ºC than at 3 ºC, while, after 13 days of storage under CO_2, the amount of L-lactic acid was always greater at 3 ºC than in samples stored in nitrogen or under vacuum for both types of poultry muscle (Kakouri and Nychas 1994). In samples stored under 20% CO_2 + 80% O_2, the L-lactate concentration decreased more rapidly than in those samples packed under CO_2, N_2 or under vacuum at either temperature (Kakouri and Nychas, 1994).

For all samples, the decrease in L-lactate was accompanied by an increase in the concentrations of acetic acid and ammonia. The extent of this increase was greater at 10 ºC than at 3 ºC. On the other hand, the increase in ammonia was always lower for samples of breast and thigh muscle stored under CO_2 (100%) at either 3 ºC or 10 ºC (Kakouri and Nychas, 1994). Nychas and Tassou (1997) found significant differences in the profiles of water-soluble proteins from poultry muscles stored under aerobic and MAP conditions. They attributed this partly to the autolytic enzymes of the meat (Schmitt and Schmidt-Lorenz, 1992a,b) and also to different degradation mechanisms associated with the predominant microbial flora in each case (pseudomonads v. lactic acid bacteria). It should be noted that, although lactic acid bacteria can produce extracellular proteinases, they are considered to be only weakly proteolytic (Law and Kolstad, 1983), when compared with e.g. *Pseudomonas* spp. However, proteolysis may provide pathogenic bacteria with suitable nutrients (Tsigarida *et al.*, 2003).

Cold-tolerant Enterobacteriaceae can be important in spoilage if the meat ecosystem favours their growth (Table 21.1). This group utilizes mainly glucose and glucose 6-phosphate as the main carbon sources and only the exhaustion of these substances will allow amino acid degradation (Gill, 1986). Moreover, some members of this family produce ammonia, volatile sulphides, including H_2S and malodorous amines from amino-acid metabolism. The Gram-positive bacteria associated with meat-storage ecosystems are also shown in Table 21.1. Among these, the physiological attributes of lactic acid bacteria and *Br. thermosphacta* have been studied extensively. Conclusions are that O_2 tension, glucose concentration and the initial pH of the medium have a major influence on the physiology of these organisms and hence on end-product formation (Nychas *et al.*, 1998). *Br. thermosphacta* has a much greater spoilage potential than lactobacilli and can be important in both aerobic and anaerobic spoilage of meat. This organism utilises glucose and glutamate as energy sources, but no other amino acid during aerobic incubation (Gill and Newton, 1977). Under aerobic conditions, it produces a range of end-products, including acetoin, acetic, *iso*-butyric and *iso*-valeric acids, 2,3-butanediol, diacetyl, 3-methylbutanal, 2-methylpropanol and 3-methylbutanol, in media containing glucose, ribose or glycerol as the main carbon and energy source (Dainty and Hibbard, 1980). The precise proportions of these end-products are affected by the glucose concentration, pH and environmental temperature (Nychas *et al.*, 1998).

21.3.3 Growth of pathogens on MAP poultry

Aeromonas hydrophila

According to the literature, *A. hydrophila* is not considered a particular hazard in meat products stored in MAP, when compared with aerobic packaging methods, given that combinations of low temperature and elevated CO_2 (>40%) concentrations are sufficient to prevent growth of this psychrotroph (Garcia de Fernando *et al.*, 1995). *A. hydrophila* was capable of growth on turkey meat stored at 1 or 7 ºC in either air or 100% N_2 (Mano *et al.*, 2000). Growth also occurred at 7 ºC in 20% CO_2 + 80% O_2, but not at 1 ºC, whereas no growth was observed in 40% CO_2 + 60% O_2 at either temperature (Mano *et al.*, 2000).

Listeria monocytogenes

The behaviour of *L. monocytogenes* on meats packaged under MAP has been studied more extensively than any other foodborne pathogen, probably because of the widespread prevalence of this organism in food-related ecosystems (Ryser and Marth, 1991), its ability to grow under chill conditions and the potential seriousness of human infections. Overall, the growth capability of the bacterium in MAP is largely dependent on the gas mixture, storage temperature, pH of the ecosystem and growth of indigenous spoilage organisms (Marshall and Schmidt, 1991; Marshall *et al.*, 1991, 1992; Tsigarida *et al.*, 2000). Nevertheless, there are conflicting reports on the growth potential of *L. monocytogenes* in meat products, including roast beef (Hudson *et al.*, 1994), lamb (Nychas, 1994), frankfurters (Krämer and Baumgart, 1992), or pork (Manu-Tawiah *et al.*, 1993) packaged in 50% CO_2 + 50% N_2, 40 CO_2 + 60% N_2 or 100% CO_2 atmospheres and stored at refrigeration temperatures. A notable characteristic of *L. monocytogenes* is its reported ability to outgrow spoilage bacteria on both raw and cooked chicken products (Wimpfhimer *et al.*, 1990; Marshall *et al.*, 1991). The natural flora probably stimulates growth of *L. monocytogenes* via proteolysis that provides readily-available nutrients and increases the pH of the food (Marshall *et al.*, 1992; Mano *et al.*, 1995). Such a situation is attributed mainly to the metabolic activities of Gram-negative bacteria on meat and poultry, which dominate in aerobic or low CO_2 atmospheres. For this reason, in studies where meat or poultry products were inoculated with the pathogen and stored in MAP at different temperatures, growth of *L. monocytogenes* occurred only under conditions that allowed abundant growth of the normal, aerobic Gram-negative flora and, generally, at a pH value above 6.0. Thus, the stimulation of *L. monocytogenes* by the endogenous flora is also dependent on meat pH, storage temperature and the gaseous environment (Marshall *et al.*, 1992). Most importantly, stimulation of *L. monocytogenes* was observed in co-culture with *Ps. fluorescens* on pre-cooked chicken stored in 76% CO_2 + 13.3 N_2 + 10.7% O_2 at 3 ºC, but not at 7 ºC, or 11 ºC, or in 80% CO_2 + 20% N_2 (Marshall *et al.*, 1992). Storage at 7 ºC and 11 ºC masked the differences in growth kinetics between the two organisms, whereas 80% CO_2 inhibited the pseudomonads. Likewise, Mano *et al.* (1995) reported growth of *L. monocytogenes* on turkey stored at 7 ºC, either aerobically or in modified atmospheres containing 100%

N_2, or CO_2 + O_2 mixtures (20% + 80%, or 40% + 60%). On the other hand, at 1 ºC, only aerobic storage and 100% N_2 allowed growth of the pathogen, albeit with a significantly lower yield. This coincided with the aerobic flora reaching the highest possible levels by the end of storage. The same MAP gas mixtures inhibited growth of *L. monocytogenes* on pork at both temperatures, due apparently to the lower initial pH (5.3) of pork compared to that of turkey (6.3).

The role of product pH in the growth of *L. monocytogenes* on meat is further supported by the findings of Barakat and Harris (1999). In their study on cooked chicken legs, they observed growth of *L. monocytogenes* at 3.5 ºC under 44% CO_2 + 56% N_2, regardless of the presence or absence of the natural flora. The pH of the product was not affected by growth of the indigenous flora, which comprised mainly lactic acid bacteria, and remained stable at around pH 6.3. However, the composition of the gaseous environment in MAP is also important in relation to the growth response of *L. monocytogenes* on poultry, since elevated CO_2 concentrations and low O_2 are inhibitory to *L. monocytogenes*, regardless of pH. Wimpfheimer *et al.* (1990) found that *L. monocytogenes* failed to grow on raw chicken stored in 75% CO_2 + 25% N_2 at 4, 10 or 27 ºC, but grew aerobically and in a 72.5% CO_2 + 22.5 N_2 + 5% O_2 atmosphere at all three temperatures. In a comparative study of two MAP systems and growth of *L. monocytogenes* and *Ps. fluorescens* on pre-cooked chicken nuggets stored at 3, 7 and 11 ºC, Marshall *et al.* (1991) found that the inhibitory effect of gaseous atmosphere on both organisms followed the order: 80% CO_2 + 20 N_2 > 76% CO_2 + 13.3 N_2 + 10.7% O_2. The effectiveness of MAP increased as the temperature was reduced. Similarly, storage of chicken in an atmosphere lacking O_2 (30% CO_2 + 70% N_2) did not permit growth of *L. monocytogenes* at either 1 ºC or 6 ºC (Hart *et al.*, 1991).

Yersinia enterocolitica

Y. enterocolitica is another psychrotrophic bacterium that is capable of growth in low oxygen tension conditions and at refrigeration temperatures in ready-to-eat meat products (Manu-Tawiah *et al.*, 1993; Hudson *et al.*, 1994). The tolerance of *Y. enterocolitica* to CO_2 appears to be higher than that of other pathogens (Garcia de Fernando *et al.*, 1995). For instance, *Y. enterocolitica* had a greater growth potential than *L. monocytogenes* on a cooked chicken product stored in 44% CO_2 + 56% N_2 (Barakat and Harris, 1999). Based on *in vitro* studies, CO_2 concentrations greater than 40% appear to have an inhibitory effect on the growth of *Y. enterocolitica* (Zee *et al.*, 1984). With regard to food products, there are contradictory reports on the CO_2-tolerance of *Y. enterocolitica*; however, the majority of studies on the growth of this pathogen on MAP foods focus on red meats (lamb, pork and beef) and there is a lack of information with respect to poultry. According to the literature, it is the combined effect of CO_2 and growth-limiting temperatures (below 3 ºC) that inhibits growth of *Y. enterocolitica* on MAP meats (Farber, 1991; Hudson *et al.*, 1994; Garcia de Fernando *et al.*, 1995). For example, studies have shown that an atmosphere of 80% CO_2 + 20% O_2 does not support growth of *Y. enterocolitica*

in minced beef during storage at 4 ºC, in contrast to 10 or 15 ºC, which allow growth similar to that observed in aerobic storage (Kleinlein and Untermann, 1990). Likewise, growth of *Y. enterocolitica* was not detected at 0 ºC in lamb packaged in 100% CO_2 or in 20% CO_2 + 80% O_2, but growth in these atmospheres was evident at 5 ºC (Garcia de Fernando *et al.*, 1995). Furthermore, some temperatures up to 20 ºC allowed growth of the pathogen on meat regardless of the highly inhibitory effect of 100% CO_2 (Eklund and Jarmund, 1983), whereas, on high-pH beef, 100% CO_2 was insufficient to prevent growth of *Y. enterocolitica*, even at 5 or 10 ºC (Gill and Reichell, 1989). In the latter study, growth of the pathogen was totally inhibited at 0, 2 and −2 ºC. Similarly, Hudson *et al.* (1994) found no growth of *Y. enterocolitica* on sliced roast beef packaged in 100% CO_2 and stored at −1.5 ºC, whereas, at 3 ºC, the bacterium showed approximately a thousand-fold increase, although growth was less than that under aerobic conditions. Tolerance to CO_2 and rapid growth of *Y. enterocolitica* have also been reported for pork chops stored at 4 ºC in modified atmospheres comprising 20% CO_2 + 80% N_2 and 40 CO_2 + 10% O_2 + 50 N_2 (Manu-Tawiah *et al.*, 1993).

Aside from the effect of CO_2, there is also evidence that the presence of O_2 in MAP gas mixtures may contribute to the inhibition of *Y. enterocolitica*, since a 50% CO_2 + 50% N_2 mixture has been found to support growth of the pathogen on lamb, even at 1 ºC, while, at 0 ºC, growth in the same gas mixture was detected only in minced lamb and not on whole lamb (Garcia de Fernando *et al.*, 1995). In practice, inhibition of *Y. enterocolitica* is also dependent on the dominant spoilage flora in the different MAP gas mixtures, which, in turn, exerts a competitive effect on the pathogen (Manu-Tawiah *et al.*, 1993).

In summary, the ability of *Y. enterocolitica* to proliferate on MAP meats is dependent on storage temperature, gaseous environment, pH, the competing microflora, the product itself (lamb, beef, poultry) and the product integrity, i.e. whole meat, cut portions or mince (Eklund and Jarmund, 1983; Gill and Reichel, 1989; Kleinlein and Untermann, 1990; Manu-Tawiah *et al.*, 1993; Hudson *et al.*, 1994).

Clostridium *spp.*

The fact that anaerobic conditions are required for the growth of clostridia has raised concern that MAP, and especially gas mixtures with limited residual oxygen, may favour growth of this type of anaerobe, particularly following temperature abuse (Foegeding and Busta, 1983). Among the clostridia, the main problem is the possibility of growth and toxin production by non-proteolytic, psychrotrophic strains of *Cl. botulinum* types B, E and F (minimum growth temperature: 3.3 ºC). The ability of these organisms to produce toxin is strongly dependent on temperature and substrate, although other ecological factors, such as pH, water activity, preservatives and headspace gas composition are also considered important (Sperber, 1982). However, it remains unclear whether MAP has a positive, neutral or negative effect on growth and time to toxin production by non-proteolytic *Cl. botulinum* (Fernandez *et al.*, 2001). It has been

reported that storage of cooked turkey at 4 ºC in 30% CO_2 + 70% N_2 delayed toxin production, compared with packaging in 100% N_2, whereas no marked effect of MAP was evident at higher temperatures, such as 10 ºC or 15 ºC (Lawler *et al.*, 2000). Furthermore, toxin production by *Cl. botulinum* type E has been reported for nitrogen-packed hamburger sandwiches at 12 ºC, but not at 8 ºC (Kautter *et al.* 1981). In contrast, under the same conditions, turkey or sausage sandwiches failed to support growth or toxin production, indicating a substrate effect in relation to the risk of botulism.

Given that *Cl. botulinum* is a common contaminant of seafood, there has been concern about the risk of growth and toxin production by types B, E and F in MAP fish or seafood products (e.g. salmon fillets). The risk appears to be greatest when the water activity of the food exceeds the growth-limiting level of 0.97 (due to insufficient salting) and the temperature is higher than 4 ºC (Garcia and Genigeorgis, 1987; Garcia *et al.*, 1987; Baker and Genigeorgis, 1990). Furthermore, the use of triple gas-mixtures containing of 90% CO_2 + 8% N_2 + 2% O_2 and 65% CO_2 + 31% N_2 + 4% O_2 has indicated a stimulatory effect on toxin production, in comparison with 100% CO_2 and N_2, atmospheres, for cod and whiting stored at 8 ºC, and this was more pronounced at 26 ºC (Post *et al.*, 1985). Other studies concluded that the spores of all three types (B, E and F) of non-proteolytic *Cl. botulinum* were capable of initiating growth and producing toxin in a fresh salmon-muscle homogenate (Garcia and Genigeorgis, 1987), fresh salmon fillets (Garcia *et al.*, 1987) and rockfish (Baker and Genigeorgis, 1990). This occurred in vacuum, 100% CO_2 and 70% CO_2 + 30% air, at temperatures from 8 ºC to 30 ºC, but never at 4 ºC. Growth was initiated, even from a single spore. Similarly, packaging of salmon fillets in 60% CO_2 + 15% N_2 + 25% O_2 and storing them at 26 ºC failed to prevent toxin production prior to spoilage (Hotchkiss, 1988).

With regard to the effect of MAP on the germination and growth of other clostridia, Baker *et al.*, (1986) found that, although *Cl. sporogenes* (10^4 cfu per g) failed to grow in ground poultry packaged in 80% CO_2 + 20% air at 2, 7 or 13 ºC, the initial population was maintained at higher levels than in aerobically stored samples. Likewise, concern has been expressed about the effect of MAP on *Cl. perfringens*, which is considered a natural contaminant of raw meat (Novak and Yuan, 2004). The effect of CO_2 on the growth of this pathogen is dependent on the CO_2 concentration, storage temperature and pre-treatment of the food. Although low CO_2 concentrations (5%) have been shown to stimulate outgrowth of *Cl. perfringens* spores (Enfors and Molin, 1978), concentrations greater than 75% prevented spore germination on cooked beef (Hintlian and Hotchkiss, 1987). Nevertheless, abuse temperatures (>25 ºC) may decrease the effectiveness of MAP under 100% CO_2 (Hintlian and Hotchkiss, 1987), while, in the case of beef, pre-packaging interventions, such as heating (60 ºC for 30 min) or ozone treatment (5 ppm O_3 for 5 min.) may enhance germination, outgrowth and multiplication of *Cl. perfringens* in MAP, in comparison with untreated MAP beef (Novak and Yuan, 2004).

Salmonella *spp.*

Results obtained by Baker *et al.* (1986) suggest that packaging of ground chicken in an elevated CO_2 atmosphere (80% CO_2 + 20% air) does not increase the risk of *Salmonella* growth, in comparison with aerobic storage, either at refrigeration or abuse temperatures. Similarly, *Salmonella* survived, but failed to grow at 3 ºC on chicken breasts and thighs stored in vacuum, 100% CO_2, 100% N_2 or 20% CO_2 + 80% air. At 10 ºC, however, rapid growth occurred in N_2 and CO_2 + air atmospheres, while, at the same temperature, a reduction in levels of *Salmonella* was observed in 100% CO_2 (Nychas, 1994).

Campylobacter

Campylobacters, especially the thermophilic species, are common contaminants of poultry meat due to various potential sources of infection in the live bird and the absence of decontamination treatments for carcasses (trisodium phosphate, organic acids etc.) in the European Union (Corry and Atabay, 2001). The low infective dose for live poultry, as well as the presence of the organisms in high numbers (up to 10^9 per carcass) on carcass meat, explains the public health concern; however, the relatively high minimum growth temperature (*c.* 30 ºC) minimises the likelihood of growth on meat, so that the health risk is more to do with survival of the organisms (Corry and Atabay, 2001). Survival of *Campylobacter* spp. is generally enhanced in the cold (<5 ºC) and at frozen storage temperature (−20 ºC), in comparison with ambient temperatures >10 ºC (Blankenship and Craven, 1982; Curtis *et al.*, 1995). In addition, pH and water activity also affect the survival of *Campylobacter* spp. in foods, with inactivation from these factors being more rapid at ambient temperatures (Doyle and Roman, 1981, 1982). Concentrations of NaCl as low as 1.5% are sufficient to prevent growth of *Campylobacter* in broth (Doyle and Roman, 1982; Corry and Atabay, 2001). This may explain the greater survival of *C. jejuni* on vacuum-packed beef cubes (of low NaCl content) at 20 ºC (Hanninen, 1981), when compared with vacuum-packed, processed turkey, formulated with 2% NaCl and stored at 4 ºC (Reynolds and Drayghon, 1987); however, little is known about the effect of MAP on the survival of *Campylobacter* spp. in other foods. In contrast to the above, Phebus *et al.* (1991) reported that *C. jejuni* survived better at 21 ºC than at 4 ºC on MAP turkey rolls. Of the atmospheres tested in this study, 100% CO_2 allowed the longest survival of *C. jejuni*, i.e. 18 days at 4 ºC and > 48 days at 21 ºC, followed closely by 100% N_2, while CO_2 + N_2 (80 + 20, 60 + 40, 40 + 60), O_2 + CO_2 + N_2 (5 + 10 + 85), and air alone showed slightly more rapid inactivation of *C. jejuni*, irrespective of the gas mixture, at both temperatures. Furthermore, growth of *C. jejuni* on beef at 37 ºC occurred more rapidly in 85% N_2 + 10% CO_2 + 5% O_2 than in vacuum or in 80% N_2 + 20% CO_2 (Haninnen *et al.*, 1984). In contrast, *C. jejuni* died out at 4 ºC, regardless of the packaging atmosphere. Growth and death rates at 37 ºC and 4 ºC, respectively, varied between strains. Similar results were reported for MAP poultry meat (Stern *et al.*, 1986). Because of the increased survival of campylobacters in CO_2-rich atmospheres, Phillips (1998) investigated the

prevalence of *Campylobacter* spp. in MAP foods. Samples of MAP chicken and MAP turkey meat were tested for the presence of the organism in two types of samples: those examined immediately after purchase and others stored at 4 ºC and tested on the 'use by' date stated on the package. In total, *Campylobacter* spp. were detected in 90% of turkey samples and 71.25% of chicken samples held in MAP. Turkey meat was found to have the highest numbers of organisms present (5.2×10^2–3.3×10^3 cfu per g) compared with 0.2–7.2×10^2 cfu per g for chicken meat.

21.3.4 Concerns and prerequisites for the safety of MAP

Over the last two decades, the increased popularity of MAP worldwide, as a method of food preservation, has stimulated investigations on the safety concerns that this technology raises. In an early study, Hotchkiss (1988) divided the experimental approaches used in assessing the safety of MAP products into four classes:

1. inoculation of products with pathogen(s) followed by MAP and monitoring of the changes in spoilage flora and pathogen populations during storage
2. correlation between organoleptic spoilage and time-to-toxin production by toxigenic organisms, such as *Cl. botulinum*
3. predictive modelling of time-to-toxin production by different population levels of a toxigenic organism, inoculated into an MAP product and
4. determination of the log count ratio for spoilage organisms and pathogens throughout the storage period, termed the 'safety index', which is indicative of whether spoilage occurs earlier than growth of pathogens.

The reviews of Farber (1991) and Garcia de Fernandez *et al.* (1995) address the fact that apparently adverse changes in gas mixtures for MAP products do not necessarily preclude growth of foodborne psychrotrophic and facultatively anaerobic pathogens. This becomes more significant in relation to the higher pH of poultry, compared to other meats. Nevertheless, combining MAP with a low temperature is always more effective than any single factor, according to the concept of multiple hurdles in food preservation (Leistner, 1995, 2000).

Regardless of the growth potential of pathogens on MAP poultry products, a general principle that ensures the effectiveness of MAP is the use of raw materials of good microbiological quality (Farber, 1991; Davies, 1995), i.e. a low, initial microbial load. This is crucial to limit the subsequent growth of pathogens on food surfaces. There is also a need for an integrated approach to pathogen reduction for all poultry that includes knowledge of the sources of contamination and means of controlling pathogens at all stages from farming and slaughter to further processing and retail display. There are many important factors that need to be considered, especially in relation to modern, quantitative risk assessments (Buchanan *et al.*, 2000; Phillips *et al.*, 2004).

21.4 Factors affecting the use and effectiveness of MAP

Nowadays, consumer lifestyles are markedly different from those of the 1960s (Paine, 1991). The last 30 years has seen a rapid growth in supermarkets and once-a-week shopping. This has major safety implications for the food industry and especially for the meat industry, and has led to the development of new technologies. The interaction between the consumer and the food industry expresses itself in various ways. One of these is in the development and exploitation of new packaging methods. The main purposes of MAP technology are:

- Shelf-life extension: depending on the type of meat or meat product, the period during which an item can remain on display in a supermarket or shop can even be doubled by using MAP techniques. This means that waste is minimised and ordering and re-stocking are more flexible.
- Enhanced appearance and presentation (i.e. perceived quality) of the product: the use of MAP has obvious attractions for both retailers and consumers and retailers have every opportunity to use more attractive packing. Thus, visual attributes of the product become another key aspect of MAP.
- Reduced use of artificial preservatives: consumer demand for longer-lasting products can be met with the aid of vacuum- or gas-packaging and without any additional, artificial preservative.
- Ability to access new markets: this can be achieved because the longer shelf-life provided by MAP allows for wider product distribution.

All of the above objectives are based on the assumption that MAP meat products are as safe as the corresponding items held under aerobic conditions and, in practice, this appears to be the case in normal usage.

In comparing poultry with red meats, it is clear that there are both qualitative and quantitative differences in the initial microflora, different proportions of unsaturated lipids and differences in muscle pigments. These lead to particular requirements for the gas mixtures to be used in MAP in order to optimise shelf-life and visual appearance (Saucier *et al.*, 2000). From the microbiological standpoint, selection of an appropriate gas mixture should take account of the higher pH of poultry, which is 5.7–6.0 for breast and 6.4–6.7 for leg muscle (Marenzi, 1986).

21.4.1 Meat quality and successful application of MAP

The application of gas mixtures containing CO_2, N_2 and O_2 is well established. Other gases, such as carbon monoxide (CO), nitrous and nitric oxides, sulphur dioxide and chlorine (Phillips, 1996), have been used for experimental purposes only. For safety, regulatory and cost reasons, these alternative gases have not been applied commercially. From the quality standpoint, meat colour is very important and selection of the gaseous atmosphere has a critical bearing on this attribute. A consumer's decision to purchase is based primarily on the colour and appearance of the product. To avoid discolouration of red meat, a high pO_2

is required so that the bright-red colour of oxymyoglobin is retained, a method called 'high-oxygen modified atmosphere' (Gill and Molin, 1991). There is, however, a relationship between O_2 and CO_2 in a meat system, meat quality and shelf-life in general. Both gases select different microbial associations from the initial contaminants (O_2 an aerobic flora and CO_2 a facultatively anaerobic flora) and both influence meat colour in different ways. A high pO_2 retains an acceptable colour, but eventually enhances fat oxidation and product rancidity (Gill and Molin, 1991). Hotchkiss (1988) suggested a mixture of 75% CO_2 + 15% N_2 + 10% O_2 for the majority of non-respiring products. In the case of poultry, it has been suggested that 10–20% CO_2 in the pack atmosphere is sufficient for a short shelf-life extension (2–3 days) at refrigeration temperature, whereas a minimum concentration of 50% CO_2 is required for a longer increase (Hotchkiss, 1989). A disadvantage of CO_2 is its deleterious effect on meat colour, due to denaturation of proteins and surface bleaching. Sørheim *et al.* (1999) investigated the effects of different gas mixtures (0.4% CO + 60% CO_2 + 40% N_2, 70% O_2 + 30% CO_2 and 60% CO_2 + 40% N_2) on the shelf-life and colour of ground beef, beef loin steaks and pork chops stored at 4 or 8 ºC. They found that the CO-containing mixture resulted in the longest shelf-life, with a longer-lasting, bright-red colour than that in a high O_2 mixture. Sante *et al.* (1993) evaluated a series of packaging atmospheres, including air, vacuum, 100% O_2, 100% N_2, 100% CO_2 plus O_2 scavenger and 25% CO_2 + 9% N_2 + 66% O_2, for their ability to increase shelf-life and maintain colour stability in chicken breast muscle. Judging by colour coordinates related to lightness, redness and yellowness, packaging under vacuum or 100% CO_2 with an O_2 scavenger significantly improved colour stability of breast muscle stored at 3–4 ºC for 21 days, compared to the other packaging atmospheres.

In addition to the commonly used gases in MAP, ozone (O_3) is another gas with established antimicrobial properties against bacteria, yeasts and moulds, and is capable of making a positive contribution to the maintenance of muscle colour (Hotchkiss, 1989; Kim *et al.*, 2003). There is an increased interest in the industry in using this gas to extend the shelf-life of MAP meat and poultry products, especially after recent approval in the USA (US Department of Agriculture) for use of ozone in food preservation and plant sanitation (Kim *et al.*, 2003).

For the successful application of MAP, the following issues are of particular importance.

1. The size and composition of the initial microflora has a pivotal role. A large initial population is unlikely to favour the successful application of a new packaging technology.
2. The timing in applying the technology is also important: the earlier the better, as far as control of aerobic spoilage organisms is concerned.
3. Strict control of storage temperature is paramount in ensuring development of the appropriate microflora.
4. The gas mixture selected must be appropriate to the product in question. Apart from other factors, maintaining the colour of the meat is a key issue.

5. The packaging materials used should be selected carefully with respect to the gases they are required to contain or accumulate *de novo*.
6. Combination processes (e.g. MAP and irradiation) or the packaging technologies themselves change the spoilage pattern of the product, and such changes need to be documented.

21.4.2 Combination of MAP with other preservation methods

It is well known that the combined use of several preservation methods (hurdle concept), as described by Leistner and Rodel (1976), is an effective means of reducing the tolerance of microorganisms to adverse environmental conditions. Under chill conditions, the concept can be applied to controlling spoilage and ensuring the safety of fresh meat and poultry, by exploiting both old and new methods of preservation. Of the several methods available for meat preservation, the most commonly used are chemical decontamination, low-dose irradiation and MAP (Lambert *et al.*, 1991). In relation to the hurdle concept, the use of MAP in combination with one or two of the other methods would increase the preservation potential of each individual method (Lambert *et al.*, 1991; Leistner, 1995, 2000; Leistner and Gorris, 1995).

Chemical agents

Many substances, including chlorine (Kraft *et al.*, 1982), short-chain organic acids (Zeitoun and Debevere, 1992), trisodium phosphate (Ismail *et al.*, 2001), herbs (Ismail *et al.*, 2001), electrolysed water (Fabrizio *et al.*, 2002; Park *et al.*, 2002) and bacteriocins have been recommended as means of reducing the microbial load on the surfaces of poultry carcasses. Although their antimicrobial properties are described in the literature, only a few (mainly organic acids) have been applied in practical situations, and even fewer have been evaluated in combination with other preservation methods, such as MAP. Importantly, lactic acid, acetic acid, propionic acid, citric acid and sorbates have been classed in the USA as Generally Recognized As Safe (GRAS) (available at http://www.cfsan.fda.gov/~rdb/opa-gras.html) and hence have attracted interest as possible decontamination agents for poultry carcasses. The following paragraphs review studies dealing with the combined effects of these chemical agents and MAP storage of poultry.

It has been reported that proper selection of CO_2 concentrations to combine with sorbates (2.5–5.0%) may reduce required levels of the latter by 50% and still succeed in inhibiting or inactivating pathogens, such as *S.* Enteritidis and *Staph. aureus*, on various foods, including fresh chicken thighs (Elliot and Gray, 1981; Elliot *et al.*, 1982; Gray *et al.*, 1984). Later, Elliot *et al.* (1985) found that a combination of potassium sorbate (up to 2.5%) and 100% CO_2, even at an abuse temperature of 10 °C, resulted in a doubling of product shelf-life, in comparison with the effect of either factor alone. Zeitoun and Debevere (1992) demonstrated that decontamination of fresh chicken legs with a sodium lactate/lactic acid buffer (pH 3.0) at concentrations of 2–10%, followed by packaging

under 90% CO_2 + 10% O_2 and storage at 6 ºC, yielded a substantial increase in shelf-life of 13 days, in comparison with untreated samples, also stored under MAP. Based on this work, the same researchers studied the effects of 10% sodium lactate/lactic acid buffer on the spoilage association of chicken legs stored under conditions identical to those of the first study, and they also monitored changes in the Enterobacteriaceae during storage (Zeitoun *et al.*, 1994). It was concluded that the greatest inhibition of microbes was achieved by combining the buffered lactic acid with MAP storage. After six days of aerobic storage at 6 ºC, the Enterobacteriaceae consisted mainly of *E. coli*, followed by the psychrotrophic organisms, *H. alvei*, *Cit. freundii* and *En. cloacae*. The beneficial effect of immersion in 10% buffered lactic acid on the shelf-life of MA-packaged chicken was further established with the use of a lower CO_2 concentration, i.e. 70% CO_2 + 5% O_2 + 25%N_2 and storage at 4 ºC or 7 ºC (Sawaya *et al.*, 1995b). Changes in the spoilage association and two other spoilage indices were monitored: extract release volume (ERV) (Egan *et al.*, 1981) and concentrations of free fatty acids (FFA). The combination of buffered lactic acid with MAP extended shelf-life by >36 and 35 days respectively at 4 ºC and 7 ºC, compared with only 22 and 13, respectively, for MAP alone. A delay in reducing ERV and increasing FFA correlated well with the shelf-life of the product under different storage conditions. In another study, pre-cooking injection of chicken legs with sodium lactate and another commercial anti-microbial, in combination with a low temperature (3.5 ºC), significantly delayed growth of Gram-positive organisms and extended the lag phase of *L. monocytogenes* and *Y. enterocolitica* in a mixture of 44% CO_2 + 56% N_2 (Barakat and Harris, 1999).

A comparative evaluation of lactic acid (1%), acetic acid (1 and 2%) and potassium sorbate (0–2.5%) as decontamination agents suggested that acetic acid would be the most effective compound for extending the shelf-life of chicken carcasses, followed by lactic acid and potassium sorbate (Tessi *et al.*, 1993). Based on these findings, Jiménez *et al.* (1999) investigated the combined effect of immersion in 1% acetic acid with packaging in 70% CO_2 + 30% N_2 and storage of chicken breasts at 4 ºC. Acetic acid treatment of chicken breasts kept total viable counts (TVC) and populations of pseudomonads, lactic acid bacteria and enterobacteria approximately 2–2.5 log units lower than in untreated samples. Moreover, sensory evaluation showed that decontaminated samples maintained a pleasant, but slightly acidic smell until the end of storage (21 days). In contrast, untreated samples developed strong off-odours early on in the storage period.

With regard to bacteriocins, nisin has been combined successfully with MAP to extend the shelf-life of poultry products (Cosby *et al.*, 1999). Addition of nisin at levels above 50 μg per ml, in combination with 20–50 mM EDTA, kept TVC from broiler 'drummettes' packaged in 20% CO_2 + 80% O_2 approximately 2 log cfu per g lower than untreated samples, after 18 days of storage at 4 ºC (Cosby *et al.*, 1999). Likewise, a combination of sakakin K with MAP exerted a strong anti-*Listeria* effect, when used for chicken breasts (Hugas *et al.*, 1998).

Indeed, addition of sakakin K (400 AU per g) or 10^6 cfu per cm^2 of sakakin-producing *L. sakei* to chicken breasts packaged in 80% O_2 + 20% CO_2 suppressed the growth of *L. innocua* at 7 ºC, compared to aerobic storage or MAP alone (Hugas *et al.*, 1998). Similar results with sakakin K have been reported for other meat products (Schillinger *et al.*, 1991).

Finally, based on a recommendation of the USDA for processors to use water containing 20 ppm available chlorine in continuous chillers (USDA, 1978), a study by Kraft *et al.* (1982) demonstrated a two-day increase in shelf-life for whole and cut-up chickens dipped in a 20 ppm chlorine solution and stored at 5 ºC. The chlorine treatment significantly suppressed growth of microbial populations; however, the shelf-life of chlorine-treated chicken was shorter than that of untreated samples stored under vacuum in a high-barrier film.

Irradiation

Irradiation (up to 10 kGy) is a preservation method that has been investigated extensively for the preservation of poultry and is discussed in detail in Chapter 19. Irradiation of chicken meat with 2.5–3.0 kGy and subsequent storage under elevated CO_2 or N_2 atmospheres at refrigeration temperatures has been shown to increase the shelf-life of poultry (Ingram and Thornley, 1959; Grant and Patterson, 1991), as well as sensitising pathogens, such as *L. monocytogenes* (Patterson *et al.* 1993; Thayer and Boyd, 1999), *S. typhi* (Lacroix and Chiasson, 2004), *E. coli* (Lacroix and Chiasson, 2004) and *A. hydrophila* (Stecchini *et al.*, 1995). For example, irradiation doses up to 1.0 kGy caused a 6-log reduction in *A. hydrophila* in ground poultry meat (Stecchini *et al.*, 1995); however, although the lethal effect of irradiation on *A. hydrophila* increased linearly with irradiation dose (0–1.5 kGy), vacuum packaging seemed to protect the organism in comparison with aerobic packaging. This was more evident during storage of irradiated (0.5 kGy) meat, packaged in air or vacuum and held at 2 ºC. Similarly, *L. monocytogenes* was most sensitive to irradiation (0–2.5 kGy) on turkey meat packed in oxygen-permeable film, rather than in vacuum packs or other MAP (Thayer and Boyd, 1999). Counts of *L. monocytogenes* were reduced by even the lowest radiation dose (0.5 kGy), but total inactivation of the initial inoculum (10^3–10^4 cfu per g) immediately after treatment was evident only for doses of 1.5 kGy or higher. In contrast to aerobically packaged turkey, the organisms survived doses of 2.0–2.5 kGy in vacuum packs and packs containing 17.2% CO_2 + 82.8% N_2, 40.5% CO_2 + 59.5% N_2 or 64% CO_2 + 36% N_2. Although the initial reduction in *L. monocytogenes* decreased with increasing CO_2 concentration, proliferation of survivors during storage at 7 ºC was inhibited as the CO_2 concentration increased. In the case of *Salmonella*, however, Lacroix and Chiasson (2004) showed that the organism was more resistant to irradiation than *E. coli*, when present on chicken breast, and MAP reduced the dose required for the same antimicrobial effect as that achieved under aerobic conditions.

21.5 Future trends

21.5.1 Alternative methods for assessing the safety of poultry stored under MAP

In addition to the use of microbiological methods, assessment of quality and safety for MAP products may involve specific indicators (Table 21.2), which correlate microbial growth with changes in physical and physico-chemical properties of packaged foods (Jay, 1986; Mattila and Ahvenainen, 1989; Mattila *et al.*, 1990; Mattila and Auvinen, 1990a,b; Randell *et al.*, 1995; Eilamo *et al.*, 1998; Koutsoumanis and Nychas, 1999; Tsigarida and Nychas, 2001). Eilamo *et al.* (1998) correlated an increase in headspace volatiles with microbiological changes occurring during storage of chicken legs at 1–7 ºC in the presence of CO_2 (20–80%). Ethanol and dimethylsulphide were the compounds detected at the highest levels and these were strongly affected by storage time and temperature (Randell *et al.*, 1995; Eilamo *et al.*, 1998). Conversely, butane, pentane and acetone were detected at the lowest levels, but influenced mostly by CO_2 and O_2 concentrations, which, in turn, were highly dependent on film permeability.

21.5.2 New developments

Packaging makes a significant contribution to the protection of food from mechanical, chemical and even biological deterioration. Remarkable progress has been made in developing packaging materials with low permeability to different gases (films of high barrier), edible composition and incorporation of antimicrobial substances. Many of these innovations are part of the significant progress in MAP and involve 'smart', 'active' or 'intelligent' packaging films (Davies, 1995). The aim of this advancement is to maximise the potential of packaging, as the last stage of food processing before distribution, to increase the shelf-life and safety of foods. The advances also represent another aspect of multiple-hurdle technology (Leistner, 1995; Leistner and Gorris, 1995).

As mentioned above, another means of extending the shelf-life of fresh poultry and poultry products is to use packaging that comprises edible coatings of lipids, polysaccharides or proteins (Gennadios *et al.*, 1997). These films act as solute, gas and vapour barriers, while incorporation of antioxidants or antimicrobials in their composition may exert an inhibitory effect on spoilage and pathogenic organisms on food surfaces that come into contact with the packaging film (Cutter and Siragusa, 1996, 1997; Han, 2000; Devlieghere *et al.*, 2004). The benefits of this technology cover the following:

- prevention of moisture-loss during storage
- reduction of rancidity from lipid oxidation and maintenance of product appearance
- prevention of weepage that make packages unattractive and increases costs, because of the need to include absorbent pads
- control of volatile flavour-loss, or penetration by undesirable volatiles from the storage environment

Table 21.2 Typical examples of indicators used for assessing quality and safety of packaged foods

Product and gas atmosphere	Indicator system[b]	Purpose	Reference
Aseptically-packed pea and tomato soup (30 °C[a]) Headspace composition: N_2 or $O_2 + N_2$	Headspace redox indicators: (1) O_2 liquid indicator (2) CO_2 liquid indicator	Detecting growth of *Staph. aureus*, *Cl. perfringens*, *B. cereus*, *L. plantarum*	Mattila and Ahvenainen (1989)
Aseptically-packed meat products and tomato soup (30 °C[a]) Headspace composition: N_2 or $O_2 + N_2$	Headspace redox indicators (micro-strips): (1) O_2 colour powder (2) pH of bromothymol blue (sensitive to CO_2)	(1) Detecting growth of *Cl. perfringens*, *B. cereus*, *L. plantarum* (2) Monitoring package integrity	Mattila *et al.* (1990) Mattila and Auvinen (1990a,b)
Raw chicken legs (1, 4, 7 °C[a]) No headspace composition	Volatile compounds: (1) ethanol (2) acetone (3) pentane (4) dimethylsulphide (5) carbon disulphide (6) dimethyldisulphide *Analytical method*: Dynamic headspace technique	Detection of changes in aerobic plate counts and detecting growth of clostridia	Eilamo *et al.* (1998)

Table 21.2 (continued)

Product and gas atmosphere	Indicator system[b]	Purpose	Reference
Marinated chicken breast Rainbow trout	Headspace indicator: O_2 colour indicator	Detecting pack leakage	Randell *et al.* (1995)
Headspace composition: $CO_2 + N_2$ (5 °C[a])	Volatile compounds: (1) dimethylsulphide (2) acetone (3) aldehydes (4) terpenes *Analytical method*: Dynamic headspace technique		
Chicken breast Fresh meat Liquid medium Headspace composition: Air/vacuum/MAP	*Analytical method*: Fourier Transform Infrared Spectroscopy (attenuated total reflectance) – correlation with microbial counts	Non-invasive detection of proteolysis in raw meat	Ellis and Goodacre, (2001); Ellis *et al.* (2002); Goodacre *et al.* (2004)
	Analytical method: Micro-biosensor for volatiles (electronic nose)	Non-invase detection of spoilage odours	Meyer *et al.* (2002); Vainionpää *et al.* (2004)

Notes:
[a] storage temperature
[b] mode of action of indicators.

- quality and nutritional benefits, such as reduced oil or fat uptake during frying
- antimicrobial properties from incorporation of preservatives, such as nisin, organic acids or natural antimicrobials e.g. certain essential oils (Cutter and Siragusa, 1996, 1997; Skandamis and Nychas, 2002).

'Intelligent' packaging, on the other hand, can monitor the food and transmit information on its quality, while the term 'active' can be used to describe a type of packaging that changes the environmental conditions within the pack (O_2-scavenging, CO_2-formation, aroma/ethylene/off-flavour/water removal or ethanol emission) to extend shelf-life, improve safety or enhance sensory properties, while maintaining the quality of the food (Davies, 1995; Han, 2000). Since all food-packaging systems require a packaging material of some kind and include a headspace in the package, antimicrobial agents may either be incorporated in the packaging material itself and allowed to migrate into the food, or be released and evaporate into the headspace. Potential antimicrobials for the above purpose include essential oils that are volatile and are regarded as 'natural' alternatives to chemical preservatives. This 'smart' packaging approach is still under development (Han, 2000; Skandamis and Nychas, 2002; Devlieghere *et al.*, 2004; Koutsoumanis and Nychas, submitted patent; Skandamis and Nychas, unpublished results).

In addition to packaging improvements, other novel technologies include the development of detectors to monitor oxygen levels, carbon dioxide-sensitive labels (that change colour when the pack is opened or damaged) detectors of bacterial toxins and microbial growth, and time-temperature indicators to detect improper handling or storage of the product (Church, 1993; Davies, 1995).

21.5.3 MAP and predictive modelling for risk assessment

Use of mathematical models for quantifying the effects of environmental factors, (temperature, a_w, pH, preservatives, concentration of gases in MAP, etc.) on growth of spoilage or pathogenic organisms in food ecosystems, is a new discipline, termed 'predictive microbiology'. This takes advantage of the large amount of microbiological data available and, with the assistance of statistical software packages, turns them into a readily-available decision-making tool in relation to food quality and safety. More specifically, predictive microbiology allows a reliable estimation of shelf-life and an accurate assessment of the likelihood of pathogen survival or growth; hence, it contributes to the improvement of current food technologies and validation of new control strategies. Moreover, it supports food safety initiatives, either as a tool in the establishment of critical limits, corrective actions in the Hazard Analysis Critical Control Point (HACCP) system and validation of HACCP plans, or as a key feature of exposure assessment (Buchanan and Whiting, 1996; Whiting and Buchanan, 1997; Hartnett *et al.*, 2002). Finally, this discipline may assist food manufacturers and authorities to define food safety objectives (ICMSF, 1996)

and food inspection requirements, and interpret surveillance information and time-temperature data obtained throughout the chill chain.

Mathematical models can be used to describe the interactions between environmental factors and microbial responses, including growth of individual strains and competitive growth (microbial interactions). To accomplish these tasks, the main factors that influence the fate of microorganisms in specific foods and food processes need to be identified and quantified, within an appropriate range, and microbiological data collected at regular intervals on the effects of the controlling factors. With regard to MAP, the variability of microbial responses in different gaseous atmospheres highlights the need for modelling approaches. The effect of MAP on microbial survival or growth can be expressed in terms of the CO_2 concentration, which is the predominant controlling factor in MAP (given the use of high-barrier films). Then, depending on the objective, growth kinetics (growth-rate, lag-phase duration) or time-to-toxin production or spore germination can be predicted as a function of CO_2 concentration and other controlling factors, such as storage temperature or chemical preservatives. Much of the work on predictive modelling and MAP has involved fish (Lindroth and Genigeorgis, 1986; Garcia and Genigeorgis, 1987; Baker and Genigeorgis, 1990; Koutsoumanis *et al.*, 2000), red meat and red meat products (Duffy *et al.*, 1994; Farber *et al.*, 1996; Devlieghere *et al.*, 2001), in contrast to the limited number of reports on poultry (Wei *et al.*, 2001). Table 21.3 provides a list of typical models that have been developed for products stored under MAP.

The limited number of predictive models for growth of pathogens on poultry, and even less for MAP poultry, inevitably restricts the development of product-specific quantitative risk assessments (QRAs). With the exception of a risk assessment for liquid egg (Whiting and Buchanan, 1997), QRA for poultry is still in its infancy. In a recent study, Hartnett *et al.* (2002) addressed the requirements for a *Campylobacter* risk assessment for broilers, starting with a qualitative approach, based on existing prevalence data. Nevertheless, available risk assessments for ready-to-eat foods (FDA/CFSAN, 2003) that also take account of data from poultry products may be useful in assessing the safety of poultry and can serve as a basis for future risk assessments.

21.5.4 Inhibiting bacterial communication as an additional hurdle in MAP

In addition to the established principle that microbial spoilage of meat can be attributed to the chemical changes caused by ESOs, a novel hypothesis relating to the regulatory mechanisms involved in microbial spoilage has attracted considerable research interest. Chemical changes caused by Gram-negative organisms at the onset of meat spoilage are regulated by the production of molecules that allow individual cells within a bacterial population to sense each other and hence regulate specific phenotypes, including those that are responsible for the production of spoilage metabolites (Bruhn *et al.*, 2004; Smith *et al.*, 2004). The process of bacterial communication via diffusion of signalling

Table 21.3 Available predictive models for the effect of CO_2 on growth or toxin production of microorganisms in meat or poultry products stored under MAP

Independent variables (factors)			Microorganism	Substrate	Dependent variables	Models	Reference
T (ºC)	Packaging	Others					
4 8 12	100% N_2 25% CO_2 + 75% N_2 50% CO_2 + 50% N_2 80% CO_2 + 20% N_2	*Sodium lactate*: 0%, 1.5%, 3.0% a_w: 0.9883, 0.9823, 0.9722, 0.9622 adjusted with SL and NaCl	*L. monocytogenes*	Brain Heart Infusion (BHI) broth	Maximum specific growth-rate (μ_{max}; h^{-1}) Lag-phase duration (λ; h)	(1) Quadratic models for $\sqrt{\mu_{max}}$ and ln λ as a function of T, CO_2%, a_w and sodium lactate (SL) (2) $\sqrt{\mu_{max}} = a \cdot (T - T_{min}) \cdot \sqrt{(a_w - a_{w,min} \cdot \sqrt{(CO_{2max} - CO_2)} \cdot \sqrt{(SL_{max} - SL)}}$ (3) $\ln \lambda = \ln(1) - \ln(b \cdot (a_w - a_w, \min) \cdot (CO_{2max} - CO_2) \cdot (T - T_{min})^2 \cdot (SL_{max} - SL))$	Devlieghere *et al.* (2001)
0 5	Vacuum	pH 5.5–7.0 a_w: 0.993, 0.980, 0.968, 0.960, adjusted with NaCl *Sodium tripolyphosphate*: 0, 0.3% *Sodium ascorbate*: 0, 0.042% *$NaNO_2$*: 0–0.0315%	*L. monocytogenes*	Sliced, cooked meats (beef, pork, chicken, turkey)	Growth-rate (generation time per h) Lag phase duration (λ; h) Time for 3-log increase (days)	Three quadratic models for growth rate, ln λ, ln 3-log increase: (1) pH-a_w (2) a_w-$NaNO_2$ (3) pH-$NaNO_2$	Duffy *et al.* (1994)

Table 21.3 (continued)

Independent variables (factors)			Microorganism	Substrate	Dependent variables	Models	Reference
T (ºC)	Packaging	Others					
4 7 10	Aerobic atm. 10% CO_2 + 90% N_2 30% CO_2 + 70% N_2 50% CO_2 + 50% N_2 70% CO_2 + 30% N_2 90% CO_2 + 10% N_2	pH 5.5–7.0	*L. monocytogenes*	BHI broth	Maximum population increase (Nmax) Generation time (GT; *h*) Lag-phase duration (λ; *h*)	Three quadratic models for the effect of pH, *T*, CO_2% on ln GT, ln N_{max} and ln λ	Farber *et al.* (1996)
1 4 8 12 16 30	Vacuum 70% CO_2 + 30% air 100% CO_2	*Inoculum levels*: (cfu per 3g sample): 10^0 10^1 10^2 10^3 10^4	Non-proteolytic *Cl. botulinum* types B, E, F	Fresh salmon tissue Rockfish fillet Rockfish homogenate Salmon fillet Salmon homogenate Sole homogenate	Maximum time of incubation required for growth initiation and toxigenesis (*Lag period*; LP) Probability of toxigenesis (P_{tx})	Lag period as a function of *T*: Log (LP) $= a + b \cdot T$ Log (LP) $= a + b \cdot T + c \cdot T^2$ Log (LP) $= a + b \cdot T + c \cdot (1/T)$ Logistic regression for P_{tx}	Garcia and Genigeorgis (1987); Baker and Genigeorgis (1990)
5 8 12	0% CO_2 + 10% H_2 + 90% N_2 5% CO_2 + 10% H_2 + 85% N_2 50% CO_2 + 10% H_2 + 40% N_2 90% CO_2 + 10% H_2 + 0% N_2	NaCl: 0.5–2.5% pH: 5.5, 6.5	Non proteolytic *Cl. botulinum* type B, E, F	Peptone, yeast, glucose, starch	Time to growth	Quadratic model for ln of time-to-growth	Fernandez *et al.* (2001)

4 10 16 22 28 34	Air Vacuum 100% CO^2	—[a]	*Y. enterocolitica*	Cooked chicken meats	Maximum specific growth-rate (λ_{max}; h^{-1}) Lag-phase duration (λ; h)	Three sets of models for the effect of temperature on μ_{max}, and λ in each packaging atmosphere $\sqrt{\mu_{max}} = [b \cdot (T - T_{max})] \cdot \{1 - \exp[c \cdot (T - T_{max})]\}$ $\ln \lambda = p/(T - T_{min})$ $\lambda = 1/[(b \cdot T - c)^2]$	Wei *et al.* (2001)
0 4 10 15 20	Air 25% CO_2 + 75% air 50% CO_2 + 50% air 80% CO_2 + 20% air 100% CO_2	—	*Pseudomonas* spp. *Shewanella putrefaciens* *B. thermosphacta* Lactic acid bacteria	Mediterranean red mullet (λ_{max}; h^{-1})	Maximum specific growth-rate	(1) Quadratic model for the effect of T and CO_2% on $\sqrt{\mu_{max}}$ (2) $\sqrt{\mu_{max}} = a \cdot (T - T_{min}) \cdot \sqrt{(CO_{2max} - CO_2)}$ (3) $\ln \mu_{max} = \ln (\mu_{ref} - dCO_2) \cdot CO_2) + Ea^b/R \cdot (1/T - 1/T_{ref})$	Koutsoumanis *et al.* (2000)

Notes
[a] no other conditions tested.
[b] universal gas constant.

molecules is called 'quorum sensing' (Whitehead *et al.*, 2001; Lazdunski *et al.*, 2004). Such molecules, also termed 'autoinducers 1 and 2' (AI-1, AI-2), as described by Lu *et al.* (2004), are commonly acylated homoserine lactones (AHLs) and their production is induced at high population densities, especially by Gram-negative bacteria. Pseudomonads, enterobacteria, *Agrobacterium tumefaciens* and *Vibrio harveyi* were the first identified producers of AHLs (Whitehead *et al.*, 2001). This is the reason why quorum sensing is thought to be associated particularly with spoilage of fresh meat. Pseudomonads cause spoilage aerobically, when they reach levels of 10^8 cfu per g, whereas enterobacteria may yield maximum population densities that range from 10^5 to 10^6 cfu per g, depending on the packaging atmosphere. Bruhn *et al.* (2004) demonstrated that 96 of 110 isolates from vacuum-packaged roast beef stored at 5 ºC were positive for AHLs. Of these, 67 were identified as *H. alvei*, followed by 20 isolates of *Serratia* spp. Lactic acid bacteria, the predominant group, and TVC reached maxima of 10^6 and 10^8 cfu per g respectively, with Enterobacteriaceae constituting 1–10% of the total population. Although off-odours from spoiled samples were either putrid or sour, it was not possible to associate *Pseudomonas* spoilage with quorum sensing. It can be speculated that the predominance of a Gram-positive flora in MAP products limits the potential for quorum sensing between Gram-negative organisms. However, given that MAP conditions can favour the growth of enterobacteria, research is needed to identify the range of CO_2 atmospheres that prevent Gram-negative bacteria from reaching their maximum population densities and thereby reduce quorum sensing between Enterobacteria.

Many compounds, such as short-chain fatty acids, can function as signalling molecules and regulate gene expression in spoilage or pathogenic bacteria (Lu *et al.*, 2004), similar to AHLs. Their natural presence in foods or their addition in the form of preservatives may favour quorum sensing and trigger specific metabolic attributes of microorganisms, including those associated with spoilage or virulence. In the study of Lu *et al.* (2004), clear AI-2 activity was detected in vegetables, but not in meat or chicken extracts. Actually, chicken and red meat elicit an endogenous inhibition of AI-2 activity and thus may limit the occurrence of quorum sensing. Elucidation of the involvement of quorum sensing in spoilage could lead to the development of new preservatives or identification of existing ones that might serve as quorum-sensing inhibitors and hamper spoilage phenotypes of the endogenous microbial flora.

21.6 Conclusions

In summary, MAP technology ensures greater product stability, without a negative effect on safety with regard to growth of pathogens. Nevertheless, the ability of MAP to inhibit or stimulate the growth of pathogens, or simply permit their survival, is dependent on the pathogen, the storage temperature and the concentrations of gases in the atmosphere of the package. In general, concen-

trations of CO_2 or N_2 higher than 45–50%, combined with low concentrations of O_2, such as less than 5%, are effective in both extending product shelf-life and preventing pathogen multiplication. Probably, the major safety concern for MAP at present is the survival of thermophilic campylobacters, which are unable to multiply at refrigeration temperatures. Survival of these organisms is greater at low temperatures than at ambient, especially in the absence of O_2.

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22

Handling poultry and eggs in the kitchen

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22.1 Introduction

22.1.1 Background

Foodborne diseases caused by microbiological agents remain a major problem in most countries that have reporting systems, with significant increases being documented in recent years (FAO/WHO, 2002). Relatively little is known about the 95% of cases that are sporadic (FSA, 2003) or the large number that go unreported (Griffith, 2000). Given the uncertainty in the statistics, care needs to be taken in their interpretation; however, it would appear that poultry meat and poultry products are common vehicles in the spread of foodborne diseases in the UK and elsewhere (ICMSF, 1998; Wong *et al*., 2004).

Poultry meat and poultry products were implicated as food vehicles in 27% of general outbreaks of infectious intestinal disease in England and Wales in 1995 and 1996 (Evans *et al*., 1998), with egg products being implicated in an additional 27%. In many studies, poultry is recognised as an important reservoir of foodborne pathogens, especially *Campylobacter* and *Salmonella*, with poultry production being considered a primary source for these organisms (Audisio *et al*., 1999; Denis *et al*., 1999). Some studies (Kramer *et al.*, 2000) have shown that a proportion of *Campylobacter* isolates from chicken belong to the same subtypes as those associated with human disease.

Contamination rates and levels for poultry meat vary over time between countries and according to the nature and origin of the samples examined. Internationally, contamination of raw chicken with *Salmonella* has ranged between 6% and 58%, whilst contamination with *Campylobacter* has been between 28% and 83% (see Table 22.1). In the UK, contamination of raw chicken with *Campylobacter* appears to be more of a problem at present than

that due to *Salmonella*. *Campylobacter* carriage rates in broiler chickens (and dairy cattle) tend to peak in the spring and late summer (ACMSF, 2004). The likely sources of *Campylobacter* contamination in poultry and the potential for control have been reviewed (ACMSF, 2004), with the environment around the broiler house being considered particularly important. *Campylobacter* contamination rates have remained high (over 50%), whilst *Salmonella* contamination of poultry meat in England and Wales has fallen to approximately 6% overall (FSA, 2003), with regional variation in contamination rates for both pathogens. *Campylobacter* contamination rates were higher in UK-produced birds, while *Salmonella* contamination was lower than in imported poultry meat. The decrease in *Salmonella* may be due, in part, to vaccination of some poultry flocks. *Salmonella* Enteritidis PT4 became prevalent in the 1980s and has been associated with both eggs and poultry meat (CPHLS – RMD, 2002). In the near future, however, it seems possible that carriage of this organism in poultry will be superseded by that of other subtypes. Historically, the UK position has been mirrored elsewhere in Europe (Geilhausen *et al.*, 1996), although chicken samples in one Belgian study (Uyttendaele *et al.*, 1999) were more often contaminated with *Salmonella* than *Campylobacter* (see Table 22.1).

In relation to human illness, it is important to know the frequency with which a particular food is contaminated with a pathogen and the level of contamination, especially with regard to the minimum infectious dose (MID), if known. In the living bird, campylobacters are found at their highest levels in the caeca, colon and cloaca, with numbers in the region of 10^5–10^9 cfu per g of intestinal content being observed (Corry and Atabay, 2001). Levels present on poultry carcasses can exceed 10^8 cells per carcass (Humphrey *et al.*, 2001). This should be viewed in the context of an MID that may be as low as 500 cells (ACMSF, 2004). The number of *Salmonella* cells present on the surface of chicken meat is difficult to determine with available methods and therefore few studies have been carried out on this aspect (Humbert *et al.*, 1997).

Unlike poultry meat, which is typically associated with both *Salmonella* and *Campylobacter*, the main pathogen found in eggs and egg products is *Salmonella* (ICMSF, 1998). In the late 1980s and early 1990s, there was concern that eggs were a possible source of *Salmonella* food poisoning in humans (ACMSF, 1993). This was primarily due to *S.* Enteritidis PT4, which could contaminate the shell, the outside of the yolk membrane or the surrounding albumen. The yolk membrane, especially if the egg is stored above 20 ºC, can become increasingly permeable, thus allowing the *Salmonella* to invade the yolk, where it can multiply more readily. However, survey data from the UK suggest that egg contamination rates have now decreased. In an all-UK study carried out in 2003, *Salmonella* was found in only one of every 290 boxes of six eggs (FSA, 2004). This represents an almost three-fold reduction compared to 1995–1996 (England only), where one in every 100 boxes was positive. In the 2003 study, there were no significant differences in contamination rate between production systems (caged production, free-range or organic). Contamination

Table 22.1 Contamination of chicken with *Salmonella* and *Campylobacter* spp.

Reference	Location	*n*	Samples analysed	*Salmonella* +ve (proportion of total samples, %)	*Campylobacter* +ve (proportion of total samples, %)
Hood *et al.* (1988)	UK	46	Whole, raw, fresh retail chickens	n.t.	48% total sample *C. jejuni*
Roberts (1991)	UK	292	Raw, chilled and frozen whole chickens	48	n.t.
PHLS (1993)	UK	713	Raw chicken	n.t.	18
ICRT (1994)	UK	160	Raw, fresh and frozen chicken pieces and carcasses from retail establishments	36	41
ACMSF (1996)	UK	562	Whole, raw, chilled and frozen chickens from retail sale	37	n.t.
Anon. (1996)	UK	160	Raw, fresh and frozen chicken pieces and carcasses from retail establishments	20	37
Harrison *et al.* (2001a,b)	UK (Wales)	300	Whole chicken, chicken breast and skin, chicken pieces	28	68
FSA (2001)	UK	4881	Raw, fresh and frozen chicken pieces and carcasses from retail establishments	5.8 (fresh = 4%; frozen = 10.8%)	50 (fresh = 63%; frozen = 33%)
Jørgensen *et al.* (2002)	UK	241	Whole, raw chickens purchased at retail outlets	25	83
Wilson *et al.* (1996)	Northern Ireland	140	Raw, chilled and frozen retail, whole chickens	7	n.t.

Flynn *et al.* (1994)	Northern Ireland	153	Raw, fresh, retail chicken wings	n.t.	65 *C. jejuni* and/or *C. coli*
ICRT (1994)	Europe	1707	Raw, fresh and frozen chicken pieces and carcasses from retail establishments of 14 European countries	21	28
Atanassova and Ring (1999)	Germany	509	Whole, raw, fresh chickens (broiler carcasses)	n.t.	30
Geilhausen *et al.* (1996)	Germany, Holland, France	1853	Raw, fresh chicken breast	20	33
Uyttendaele *et al.* (1999)	Belgium	772	Raw chicken carcasses and products from five European countries	37	29 *C. jejuni* and/or *C. coli*
Rayes *et al.* (1983)	USA	265	Raw, fresh and frozen turkey wings from supermarkets	n.t.	62 *C. jejuni*
Kinde *et al.* (1983)	USA	94	Fresh, packaged chicken wings	n.t.	83
Rayes *et al.* (1983)	USA	265	Fresh turkey wings	n.t.	62
Stern *et al.* (1985)	USA	360	Whole, raw, fresh chickens	n.t.	30 *C. jejuni* and/or *C. coli.*

Note: n.t. = not tested.

rates for *S.* Enteritidis PT4, traditionally the most common egg strain, fell from 0.58% in 1995–1996 to 0.11% in 2003. The fall is likely to reflect measures introduced in the UK to reduce egg contamination, including vaccination, and appears to have resulted in a decline in laboratory-confirmed cases of human salmonellosis. However, the reduction in *Salmonella* contamination may not apply to all eggs that are imported into the UK.

The USA, in its attempts to tackle the problem of *S.* Enteritidis in eggs, introduced an Egg Safety Action Plan in 1999. As part of this, the Food and Drug Administration published a regulation that introduced refrigeration requirements for shell eggs at the retail level. This became mandatory in June 2001 and required restaurants, caterers, retailers, etc., to keep eggs below 7.2 ºC.

Typically, a fresh egg, contaminated internally with *Salmonella*, would contain less than 20 organisms. The chances of an individual becoming infected from such an egg, if handled correctly, would be small. However, some desserts and other items made with pooled eggs have been implicated in food poisoning outbreaks and have given greater cause for concern (Griffith, 2000).

Whether in eggs or poultry meat, pathogen contamination rates and levels become important in relation to how much of the product is consumed and the methods of preparation employed. In the pursuit of cheap, but nutritious food, consumers have turned increasingly to poultry meat and poultry products, and over 700 million chickens a year and 33 million eggs a day are sold in the UK. Thus, even low or declining contamination rates could still contribute significantly to human disease.

The responsibility for food safety belongs to everyone in the food chain (Griffith, 2000). If handled correctly, contaminated raw foods should not pose a problem and producers have the right to expect food handlers further down the supply chain to handle food hygienically. Nevertheless, food handlers can reasonably expect producers to implement all necessary measures to minimise contamination of poultry meat and eggs. This chapter examines the knowledge, attitudes and practices of food handlers within the framework of exposure assessment, risk management and risk communication.

22.1.2 Domestic and catering kitchens: the final line of defence

In England and Wales, 12–17% of general foodborne disease outbreaks (multiple cases involving members from more than one family and thought to have a common route of exposure) are reported to have originated from the home (Tirado and Schmidt, 2000), and recent European data have shown the home to be the most important single location for the occurrence of foodborne disease (FAO/WHO, 2002). The majority (>95%) of cases do not occur as outbreaks, but are ‘sporadic’ or apparently isolated cases (FSA, 2000), and therefore less likely to be identified by public health authorities (Worsfold and Griffith, 1997). Thus, the actual number of cases occurring in the home is likely to be much larger than that suggested by the reported data. Some estimates indicate that 50–87% of all cases of foodborne disease, involving general

outbreaks, family outbreaks (multiple cases, but within the same family) and sporadic cases, may be acquired in the home (Clayton *et al*., 2003).

Statistics from a number of countries, including the USA, Netherlands and the UK, show that the majority (up to 70%) of general outbreaks are associated with catering or food service locations (Griffith, 2000). Comparisons between countries, with respect to the origins of foodborne diseases, are difficult due to differences in interpretation, definitions, methodology, data collection, etc. For the UK, it has been suggested that one in every 1527 catering establishments could be implicated in an outbreak of foodborne illness in any one year (Coleman *et al*., 2000). Whether commercial or domestic, the kitchen represents the last link in the food chain and the final line of defence, i.e. the last opportunity to eliminate previous microbial contamination and prevent further contamination before the food is eaten. In this context, both poultry meat products and eggs require appropriate handling to minimise risk, and good hygiene practices should be used in all kitchens.

22.1.3 Risk factors

Various reviews have attempted to quantify the relative importance of different food-handling practices and associated risks (Griffith, 2000). Most have been undertaken by surveillance and enforcement personnel following an outbreak and involve asking food handlers about their recollection of hygiene practices that may or may not have been used. Again, direct comparisons between countries are difficult, because the methods of investigation and terminology used may vary. One of the most variable risk factors in the different studies is cross-contamination, some studies implicating it in only 5% of cases, others in as many as 39% (Griffith, 2000). Part of the variability can be explained by differences in methods of data collection, although it is highly likely that behavioural details relating to cross-contamination are usually forgotten or unknown. As such, cross-contamination is likely to be under-reported (Griffith, 2000) and observational studies (Worsfold and Griffith, 1997; Redmond *et al*., 2004; Clayton and Griffith, 2004) indicate a much greater potential or threat from cross-contamination for some pathogens than epidemiological data may suggest.

In the UK, cross-contamination was implicated as a risk factor in 39% of general outbreaks (Evans *et al*., 1998). However, the importance of cross-contamination is likely to vary with the pathogen and its characteristics, including survival capability, transfer rates, MID, as well as the frequency and level of contamination in different foods.

Raw foods in kitchens, especially commercial ones, often undergo a lengthy series of preparative steps before becoming part of a more complex dish. Catering and domestic kitchens, unlike larger manufacturing units, are often subject to contamination from a wide variety of sources, including many types of raw food, yet may lack strict segregation between raw- and cooked-food handling areas. Domestic kitchens may be subjected to an even wider range of

contaminants from sources such as dirty laundry and pets (Worsfold and Griffith, 1997). The latter may be of particular concern in relation to *Campylobacter* (ACMSF, 2004). Pathogens can spread rapidly in kitchens and have been isolated from numerous sites in commercial and domestic kitchens, including working surfaces (Griffith, 2000).

Other reported risk factors include inadequate cooking, which, traditionally, has been seen as the main risk associated with poultry meat and eggs. Cooking is a step in much domestic and commercial food preparation, and makes the food more attractive, digestible and, above all, safe. However, consumers preparing food in the domestic kitchen may select cooking times and temperatures by means of guesswork. This can be particularly true for the cooking of poultry carcasses at Christmas time, when the carcasses are usually larger than normal. Commercial kitchens are more likely to utilise probes or other thermometers to monitor the cooking process and have food safety programmes in place, so that cooking times and temperatures should be more controlled than in domestic kitchens. This may not be the case with some desserts and sauces, the production of which can involve lightly-cooked eggs. Insufficient heat processing that is inadequate to destroy *Salmonella* can be a problem with this type of dish in both commercial and domestic kitchens.

One difference between food manufacturing and food service establishments is that the latter normally produce to order rather than using stock. However, in event and function catering, food is often prepared for unusually large numbers of people in advance of consumption, without access to additional workspace or refrigerated storage facilities. Coupled with the length of time that elapses prior to consumption, this can combine with other risk factors and contribute to food-poisoning outbreaks. Preparation of food in advance of consumption has been reported as a risk factor in many countries and has been implicated in over 50% of general outbreaks in the UK (Griffith, 2000).

22.2 Raw poultry meat and eggs: food handlers' knowledge, attitudes and practices

22.2.1 Background

Studies have suggested that improper food-handling practices contribute to approximately 97% of foodborne disease incidents arising from food service establishments and the home (Howes *et al*., 1996). Consequently, in order to reduce foodborne disease, it is crucial to gain an understanding of the interaction between prevailing food safety beliefs, knowledge and practices of food handlers. Given the potential for contaminating food and the reported role of food handlers in causing foodborne disease, their behaviour is especially important in the preparation of poultry meat and egg dishes. It is critical to store foods properly, heat them adequately (to destroy any pathogens present) and minimise any cross-contamination of other foods, equipment and working surfaces with pathogens from poultry meat or eggs.

22.2.2 Caterers

The way in which caterers perceive the importance of the food hygiene knowledge they possess can influence their intentions and practices. Unfortunately, caterers often fail to realise the risks associated with their businesses (Clayton *et al*., 2002). One study of caterers' attitudes showed 12% felt that no great care was required in handling poultry meat and 14% had no reservations about serving only lightly-cooked eggs (Coleman *et al*., 2000). In other research, caterers have admitted that they often fail to implement all the food safety procedures they know (Clayton *et al*., 2002). Studies based on detailed observation of caterers handling raw poultry meat (Clayton and Griffith, 2004) confirmed failures in implementing hygiene practices, especially those concerning the prevention of cross-contamination. These deficiencies included failure to take appropriate decontamination steps, such as handwashing and cleaning of surfaces, etc., after handling raw poultry. Barriers preventing the use of food safety procedures in catering have been reported and are mainly associated with lack of sufficient time and personnel (Clayton *et al*., 2002).

One of the challenges facing governments and the food service industry worldwide is how to introduce the Hazard Analysis Critical Control Point (HACCP) system, into catering (Mortlock *et al*., 1999). The system has been shown to be effective in improving the microbiological quality of food, and widening HACCP implementation in the catering industry will be increasingly important with intended new European Union regulations (Worsfold and Griffith, 2003). Caterers are not usually hostile to the HACCP system, although there are concerns that many businesses may not have appropriate hygiene practices in place to underpin any HACCP plans. In the UK, the Food Standards Agency (FSA) is using a strategy based on a 'jargon-free' approach to implementing the HACCP system in catering operations; this is known as 'Safe Food: Better Business'.

22.2.3 Consumers

A review of 88 international studies on consumer food safety knowledge, attitudes and practices has indicated the need for improvement (Redmond and Griffith, 2003). Knowledge, attitudes, intentions and self-reported practices did not correspond to observed behaviour. Consumers lacked knowledge of certain key issues, e.g. need for cooling of cooked food, while other hygiene practices were recognised, but specific requirements for their adequate implementation were poorly understood. *Salmonella* was invariably recognised as a potential pathogen, although relatively few consumers were familiar with the name *Campylobacter*. This is of concern, given the frequency and levels with which poultry is contaminated with the organism. Overall, consumers were aware of the generic need to cook foods properly in order to reduce the risk of foodborne disease. In many cases, however, they were ignorant of how this could be achieved and monitored for different food products and cooking methods.

Consumers often failed to appreciate the importance of the home as a location for acquiring food poisoning and generally believed that their own hygiene skills

were adequate (demonstrating the illusion of control), while other peoples' practices presented a greater risk (Redmond and Griffith, 2003). Although some studies showed evidence that consumers may accept the view that food safety is a shared responsibility, numerous observational studies (Redmond *et al.*,, 2004) have indicated a common lack of good hygiene behaviour in the handling of both poultry meat and eggs. The extent of undercooking varied with the method of cooking used, while the risk of cross-contamination from the handling of raw poultry meat and eggs was considered high.

22.3 Poultry handling and risk assessment

25.3.1 Background

Risk assessment is a science-based type of investigation consisting of four steps: hazard identification, exposure assessment, hazard characterisation and risk characterisation (Griffith *et al.*, 1998; Lammerding and Fazil, 2000). Microbial risk assessment (MRA) is concerned with the hazards presented by foodborne pathogens or associated toxins and attempts to determine the levels of risk associated with particular foods (Voysey, 2000). All forms of risk assessment usually involve some degree of quantification, ranging from the simple categories of high, medium or low risk to detailed mathematical calculations. A number of quantitative risk models have been developed to assess the risk throughout the food chain associated with specific hazards, such as *S.* Enteritidis in eggs and salmonellas in frozen chicken (Brown *et al.*, 1998; Anon., 2000; FSIS, 1998). These detailed risk assessments attempt to take account of variability (heterogeneity and diversity within systems) and uncertainty arising from imperfect knowledge (Dennis *et al*., 2002). The effect of the former cannot be reduced by additional knowledge, the latter can. One area of variability and uncertainty relates to the dose-response relationship. This is a simplified representation of the complex relationship between a dose and the adverse effect caused by a pathogen (Dennis *et al.*, 2002). Dose-response relationship may vary considerably between strains of a pathogen (different potential for virulence) and between affected individuals (different host susceptibility). Most dose-response modelling has used empirical models, often involving data from young, healthy individuals, but this can be improved by the use of mechanistic models (Dennis *et al.*, 2002). Another topic, and possibly one with the greatest uncertainty and variability, relates to exposure assessment in food service establishments and the home (Anon, 2000).

22.3.2 Exposure assessment

Exposure assessment is an estimation of the likelihood that an individual or population will be exposed to a microbial hazard and the number of organisms likely to be ingested (Lammerding and Fazil, 2000). Accurate exposure assessment requires data on the occurrence of the hazard in raw ingredients, the

circumstances under which the food is prepared and consumed, and the consumption patterns involved (Griffith *et al.*, 1998; Lammerding, 1999). This type of data is more likely to be available for manufactured food items than for foods consumed in the home or in food service establishments. It is no surprise, therefore, that the majority of risk assessment models are based only on data obtained from the farm through to retail level (Anon., 2000).

It is important to identify exposure pathways and assess their associated risk in order to inform both risk management and risk communication. When food-service establishments or domestic food-preparation areas have been considered in the past, risk was usually associated with the adequacy of cooking. The need for data on cross-contamination in the kitchen environment and re-contamination of cooked and ready-to-eat foods has largely been neglected (Anon., 2000). Furthermore, cross-contamination is likely to be under-reported in the documented incidence of foodborne disease (Griffith, 2000). Most information available on domestic food handling is based upon self-reporting, and this may be misleading, with actual practices being quite different (Redmond and Griffith, 2003). An additional problem is that foodborne disease often results from a combination of errors or malpractices associated with specific foods, and this is not usually determined in self-reporting (Griffith *et al.*, 1998). Direct observational studies, in conjunction with microbiological testing, offer a more reliable data source in order to inform exposure assessment and hence the risk associated with food handling and preparation by consumers or caterers (Redmond and Griffith, 2003).

22.3.3 Exposure pathways

Figure 22.1 represents the possible exposure pathways by which *Salmonella* or *Campylobacter* could gain access to the consumer from raw chicken. This model requires knowledge not only of product contamination rates and levels for both pathogens, but also that from the associated packaging material. Data and degree of variability is readily available for the former (Table 22.1), but less so for packaging materials, although some studies have indicated that packaging could be a source of pathogens within the kitchen. In the UK, the rate for *Campylobacter* ranged from 3% (Harrison *et al.*, 2001a) to 6% (Jørgensen *et al*., 2002), with lower rates for *Salmonella*. More recently, data from New Zealand showed contamination rates for external packaging of 24% for *Campylobacter* and 0.3% for *Salmonella* (Wong *et al*., 2004). External contamination of raw chicken packaging has implications for food handlers in retail stores as well as in food service establishments and the home. Overall contamination of packaging material, taking both outer and inner surfaces (where the latter is in direct contact with the product), can be higher, up to 34% for *Campylobacter* and 11% for *Salmonella* (Harrison *et al*., 2001a).

The cooking stage for raw poultry meat is a Critical Control Point (Dillon and Griffith, 2001), and whether or not it is carried out correctly is likely to depend on the method of cooking used (Clayton and Griffith, 2004; Harrison *et al*.,

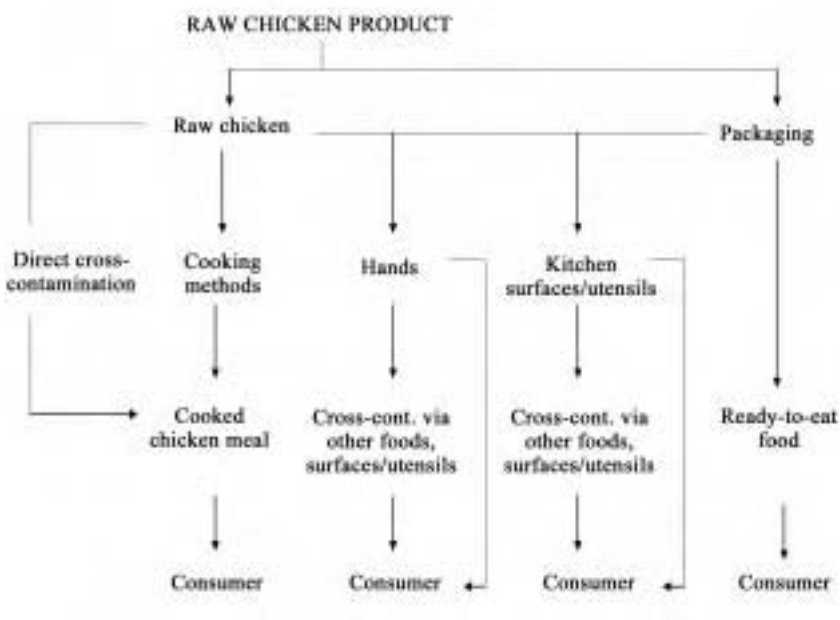

Fig. 22.1 Simple representation of exposure for pathogens transmitted from the handling of raw chicken.

2001b). Research has shown that domestic preparation of some chicken meals, such as curries and roast dinners, means that these are more likely to be cooked thoroughly and will ensure pathogen destruction. Other cooking techniques employed in the home, such as stir-frying and the use of barbecues, were more likely to allow survival of pathogens (15% and 20% undercooked respectively).

Table 22.2 illustrates some findings of cross-contamination research, indicating the spread of pathogens, such as *Campylobacter*, around a kitchen during handling of raw poultry meat and other foods by consumers and professional food handlers. Prior to meal preparation, only the poultry meat had been positive for *Campylobacter* and *Salmonella*, with all other foods and work surfaces being negative. This and other findings (ACMSF, 2004) highlight the need for careful handling of food, effective cleaning of surfaces, etc., and handwashing in kitchens.

22.4 Educating food handlers

Whether educating consumers or professional food handlers, the message (i.e. what is to be communicated), the method(s) of communication and the social and environmental context of the food handler need to be considered.

Table 22.2 Microbiology of foods and kitchen surfaces after handling and cooking raw poultry meat

	Caterers	Consumers
Cooked chicken		
+ve *Salmonella*	0%	0%
+ve *Campylobacter*	6%	8%
Other foods (salads)		
+ve *Salmonella*	0%	0%
+ve *Campylobacter*	8%	7%
Surfaces		
+ve *Salmonella*	0%	0%
+ve *Campylobacter*	12%	20%

Initial levels of contamination for raw poultry were 29% for *Salmonella* and 68% for *Campylobacter*. All other foods and surfaces were negative for *Salmonella* and *Campylobacter* prior to preparation.

The requirements of good hygiene practice are well described (Worsfold and Griffith, 1997; Redmond and Griffith, 2003), yet excessive generalisation, misunderstanding and confusion remain about key elements. For example, the washing of raw foods, as recommended by the Institute of Food Science and Technology (IFST, 2004) would increase the potential for cross-contamination of poultry meat, spreading *Campylobacter*, and other pathogens around the sink area (Griffith *et al*., 1999).

22.4.1 Guidelines

Hygiene guidelines for poultry meat and eggs (see Table 22.3) usually consist of a list of 'do's and don'ts', and what is often missing is the requirement for food handlers to think first about the practices they will use, i.e. an element of planning is required for hygienic handling, and raw poultry meat should be treated with respect. For example, if both hands are used or needed to unwrap the pack, then clean, hot soapy water should already be available in a sink or bowl for handwashing. Alternatively, unwrapping poultry portions can be undertaken, with relatively little additional effort, in such a way that only one hand is in contact with the raw meat and becomes contaminated. This leaves the other hand free to touch taps or appliances, thus avoiding contamination of kitchen surfaces. Given the high initial levels of microbes on raw poultry meat and possible transfer rates of 10%, care needs to be taken to minimise the spread of any pathogens present during handling. Some individuals take more than three times the number of food-handling steps as others to prepare the same meal. The greater the number of activities, the greater the opportunity for cross-contamination, which could, in turn, be reduced by a little forethought and care.

Although guidelines recommend cleaning of the kitchen after the handling of raw foods, this is often done poorly in both the home and commercial kitchens. Microbial counts from working surfaces, etc., are often higher after cleaning

Table 22.3 Guidelines for handling eggs

Buy from reputable supplier	Look for quality assurance mark and country of origin
Check 'best before' date	Eggs that are fresh have less likelihood of pathogen multiplication
Store in a cool, dry place and handle below 20 ºC	Check temperature in retail display, commercial transport and refrigerator in kitchen. Reduced temperature minimises bacterial multiplication
Store separately from other foods	Contamination of the shell can occur with pathogens that may spread to other foods
Wash hands before and after handling	This includes use of hot, soapy water, rinsing and drying (NOT passing hands or fingers under running tap water)
Do not use dirty or cracked eggs	Visual assessment. Cracked shells increase the chances of egg contents being contaminated with pathogens
If not all consumed at first sitting, chill prepared foods, e.g. desserts, quickly and hold refrigerated	Rapid cooling minimises opportunities for microbial growth and covering the food prevents recontamination
Do not eat or serve raw, lightly-cooked eggs	This should apply to everyone, BUT some people like runny yolks and feel there is little risk. Whilst risk is probably low, they need to accept the risk and responsibility. Raw or lightly-cooked eggs are NOT recommended for pregnant women and other vulnerable groups
Take particular care with desserts or sauces made from pooled eggs	Ensure thorough cooking of composite egg dishes to ensure pathogen destruction or, alternatively, used pasteurised eggs
When preparing eggs (especially whisking or similar activities) ensure surrounding areas, utensils and other equipment are well cleaned afterwards	*Salmonella* can survive, especially in small particles of yolk and albumen. Contaminated egg droplets can easily spread

than beforehand (Griffith *et al*., 2002). Reasons for this include insufficient time for disinfectants to function properly, use of contaminated water, cloths or equipment and failure to rinse adequately.

22.4.2 Educating food handlers: training

Education and legislation have been advocated as part of a dual strategy to reduce food poisoning. Current UK legislation now contains a requirement for food handlers to be trained or supervised in accordance with their work activities. This was introduced in 1995, but, although industry considers training

important, levels of training are variable, particularly with regard to different types of employee and food industry sector (Mortlock *et al*., 2000). The food service sector, in particular, lags behind, and there may be specific reasons for this. For example, it is recognised that there are many part-time food handlers, there is a high turnover of staff in some businesses and, for many, English is not their first language (Griffith, 2000).

Although more than four million people have been trained already, it has been estimated that this figure represents only 46% of all food handlers. However, even if food handlers are trained, there is no guarantee that their behaviour will always ensure that hygiene requirements are satisfied. Generally, there is a lack of information on the efficacy of staff training, although individual studies have produced mixed results, some showing an improvement in knowledge and others no improvement (Clayton and Griffith, 2004). In cases where knowledge improved, this did not necessarily change behaviour. Therefore, it has been suggested that training should include consideration of behavioural theory (Griffith, 2000). Continuing high levels of foodborne disease (Mortlock *et al*., 1999), coupled with repeated, basic errors in food handling, should act as a spur to make learning more effective and realise the need for improved food safety attitudes and culture.

Most studies of food hygiene learning have concentrated on the formal approach, as opposed to work-based learning (defined as learning at work, linked to a job requirement), although the two differ. Companies should try to develop training strategies that embrace both types of learning. Suggestions have been made for improving the quality of current formal courses, including changes to syllabuses, examinations and pass marks, as well as recognising the need for more HACCP training. The legislation itself has also been criticised. At present, the proprietor of a food-service establishment need not necessarily be trained and, whilst guides to the implementation of legal requirements provide a definition of a 'food handler', they are not themselves legal documents.

The following should be considered in purchasing or delivering formal training.

- Training should be targeted, specific and geared to the needs of the audience, using work-related examples.
- Information should be delivered within a hazard and risk framework.
- The content should be simple, accurate and jargon-free, delivered at an appropriate level that would promote understanding. It is important to cover both practice and theory – what to do and why.
- Trainers should have the respect of the trainees, i.e. be considered reputable and trustworthy, and have 'street credibility'.
- The trainer should examine, with trainees, likely barriers to implementation of good practice in their workplace and how these can be overcome.
- Trainers should assess available facilities and equipment, and ensure that implementation of good practices is possible. If inadequate, the management should be informed.

- The correct management 'culture' should be in place. Trainees must work in an environment where implementation of all good practices is the norm and inappropriate practices are not tolerated.
- A motivational framework should be provided to encourage training and implementation of learned behaviour.
- Training should address costs (including those of failure) as well as benefits. The latter may be financial, social or medical.
- The regular training programme requires updating periodically and the maintenance of training logs and records.
- The effectiveness of training should be evaluated as appropriate and improvements made where necessary.

22.4.3 Educating consumers: health education and social marketing

Until recently, educating the consumer about food hygiene has been largely ignored. The publicity concerning food poisoning in the UK in the late 1980s led to the production of different leaflets that provided generalised food safety advice for consumers. However, findings from other areas of health education suggest that, whilst leaflets can play a role in raising awareness, generally, they do not result in any behavioural change. Understanding the behaviour of food handlers is a complex process, with the use of behavioural scientists being recommended (Griffith *et al*., 1995) and subsequently used (Clayton *et al.*, 2002; Clayton and Griffith, 2004). Studies have been undertaken on the value of different sources of food safety information for the consumer (Griffith *et al*., 1994; Redmond *et al.*, 2000), including TV, press, etc. Leaflets can provide people with information that will enable them to change, if they are motivated to do so. The largest, coordinated food safety campaign for UK consumers is National Food Safety Week, first launched by the Food and Drink Federation some ten years ago.

In many countries, studies on consumer aspects of food safety have mostly relied upon self-reporting of behaviour. Fewer studies have focused on actual behaviour and, when these have been carried out, they have shown that consumers often do not know or understand hygiene principles and may not implement known hygiene practices when they do understand (Redmond and Griffith, 2003).

Difficulties in improving food safety practices among consumers have been reviewed and more recent attempts, using a social marketing approach, have had some success in changing behaviour (Redmond *et al.*, 2000). Social marketing is the application of marketing techniques used in the commercial sector, to promote voluntary changes in socially important public health behaviour, e.g. drug use, smoking. Social marketing is a consumer-orientated approach that starts and ends with the target customer – the person whose behaviour needs to be changed. This requires the use of formative research, a careful evaluation of consumer behaviour and the identification of target risk groups, using audience segmentation. A precise message is identified and the response to and beliefs

Table 22.4 The '4 Ps' of marketing (marketing mix) in relation to changing the behaviour of food handlers

Product	What is being offered must be acceptable and as tangible, accessible and attractive as possible. The changed behaviour must not be excessively expensive or time consuming to achieve, nor should it be in any way impracticable, painful, etc.
Price	Decisions to act result from a consideration of both costs and benefits. Ways to minimise the costs as well as maximise the benefits need to be addressed in all strategies for behavioural change.
Place	The means to implement new behaviour must be readily available and conveniently placed, e.g. availability of soap for handwashing.
Promotion	The message needs to be 'advertised' in the most appropriate way. This could include leaflets or via TV, cookbooks, magazines or individual advice, e.g. from health visitors.

about the message among the target audience are assessed, as well as the likelihood that individuals will change their behaviour. Results from these preparatory stages are then used to plan an initiative based upon marketing principles and involving the '4 Ps' or marketing mix (see Table 22.4). Social marketing initiatives are evaluated during planning and development, as well as after the implementation of the initiative.

Since the aim of social marketing is to achieve voluntary behavioural change, observation of targeted behaviour to evaluate initiative techniques is a preferable method of summative evaluation. This type of approach is more comprehensive and expensive than traditional approaches used in health education. Generally, the latter are more concerned with a top-down planning approach, imparting and receiving fact-based messages to increase consumer knowledge. Social marketers argue that an increase in knowledge is only important if it results in the desired, voluntary, behavioural change. Whilst there are differences between marketing commercial products and improving food handling, not least in terms of available budgets, there are potential benefits in adopting the marketing approach. Relatively small improvements in behaviour could significantly reduce the incidence and cost of foodborne disease.

22.5 Future trends

Poultry meat and eggs are relatively cheap, versatile and nutritious food products, and the demand is likely to increase further. Producers have a responsibility to ensure that these products are as free as possible from pathogens during production. However, a substantial proportion of all food poisoning is related to deficiencies in food service establishments and the home, and the role of food handlers and associated good food-handling practices, is paramount. Legislation for commercial organisations is becoming more stringent. This does

not normally apply in the home; however, elements of good kitchen practice that are relevant to the home should be linked to risk-based consumer advice as part of public health protection.

Achieving desirable food handling practices is likely to be of major importance to UK bodies such as the FSA, although this is only partly dependent on providing the relevant information. Good food handling requires an understanding of the factors that influence human behaviour, and greater efforts need to be made to promote good handling practices, including those of consumers. There have been considerable achievements with respect to food safety training in the UK, but further improvements are required. A greater percentage of the workforce in the food industry requires training, and this training needs to be made more effective. In the future, more training will be needed, particularly with respect to the HACCP system; also, it is paramount that individual businesses provide the correct cultural framework and context for food safety to be practised. It is not only a food company's obligation to provide training, but also to allow the learning to be implemented in the workplace. Ultimately, failure to do so could cost a business money or even its survival; however, of possibly greater importance is the fact that it may cost someone their life.

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Index