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Poultry meat pathogens and its Control

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Abstract

Poultry meat can be contaminated with a variety of foodborne pathogens that may cause human illness following ingestion and is due to handling of raw meat, undercooking or mishandling of the cooked product. While *Salmonella* and *Campylobacter* spp. remain the organisms of greatest global concern, others present include the more recently reported *Arcobacter* and *Helicobacter* spp. and, occasionally, verotoxigenic *Escherichia coil.* Also considered here is the growing problem of antimicrobial resistance among poultry-associated pathogens. Because of the need for a systematic and universally applicable approach to food safety control, the Hazard Analysis Critical Control Point (HACCP) concept is increasingly being introduced into the Poultry Industry, and Quantitative Risk Assessment(QRA) is being developed. Among a number of completed and on-going studies on QRA are those undertaken by FAO/WHO on *Salmonella* and *Campylobacter* in broilers. In the case of *Campylobacter,* however, any QRA must assume at present that all strains have the same pathogenic potential for humans, even though this is unlikely to be the case. Implementation of the HACCP system in poultry processing plants addresses zoonotic agents that are not detectable by conventional meat inspection procedures. The system brings obvious benefits in optimizing plant hygiene, ensuring compliance with legislation and providing evidence of 'due diligence on the part of he processor. It is now being applied globally in two different situations: in one, such as that occurring in the USA, carcass contamination is progressively reduced as carcasses pass through the process and are finally chilled in super-chlorinated water. There is also the option to use a chemical-rinse treatment for further reduction of microbial contamination. In the second scenario, processors in the EU are not allowed to super-chlorinate process water, and water chilling, which has an important washing effect, is confined to carcasses intended for freezing. Also, chemical decontamination is prohibited until 2006 at the earliest. Therefore, for fresh carcasses that are air chilled, there is presently no progressive reduction in carcass contamination and no Critical Control Point at which a significant reduction in pathogen contamination can be guaranteed. Overall, effective control of the organism is best realized through a farm-to-fork approach at all stages of the supply chain.

Keywords*:* Poultry meat, processing, microbial pathogens, controls.

Introduction

Contamination of poultry meat with foodborne pathogens remains an important public health issue, because it can lead to illness if there are malpractice in handling, cooking or post cooking storage of the product. In developed countries, foodborne illness causes human suffering and loss of productivity, and adds significantly to the cost of food production and healthcare. It is also a possible cause of mortality, which is even more of a problem in developing regions, where the health status of many individuals is already compromise. Numerically, the most important agents are *Salmonalle* and *Campylobacter* spp. Data for the European Union (EU) show that in 2001, there were 157 822 reported cases of human salmonellosis and 156232 cases of *Campylobacter enteritis* (Cavitte, 2003), although both diseases are known to be under-reported, and true figures are likely to be considerably higher. While poultry is by no means the only sources of the causative organisms, it is widely recognized as a major reservoir in each case, due to symptomless carriage in the live bird (Table 1). The problem is exacerbated by modern conditions of intensive rearing, where large number of birds are kept together, and high-rate processing, in which carcasses remain in close proximity throughout the operation. Such conditions favor the spread of any pathogens that may gain access to the flock. Moreover, usage of antimicrobials in poultry production, where for prophylactic, therapeutic or performance-enhancing purposes, contributes to the development of resistance in pathogens, which is increasing, and can have serious consequences for the treatment of human illness from these organisms. With salmonellosis, for example, the testing of 27 000 isolates from human cases in ten European countries in 2000, showed that almost 40% were resistant at least one antimcrobial, while 18% were multiresistant (Threlfall *et al*., 2003). Multiple resistance was most often observed in serotype Typhimurium, including DTs 104 and 204b, and 51% of Typhimurium strains were in this category. Serotypes from human with multiple resistance include those that also found in poultry, of which *S. paratyphi* B variant Java is the most recent example. In the Netherlands, variants Java had increased in poultry from less than 2% of isolates before 1996 to 60% in 2003 (Van Pelte *et al.,* 2003). The resistance of *Campylobacter* to antimicrobial is also rising, especially to fluoroquinolones , which are widely used in both human and veterinary medicine.

Table1.1 Feature of Intestinal carriage in *Campylobacter* and *Salmonella* spp.

Feature	***Campylobacter***	**Salmonella**
Host susceptibility	Not age-related	Age-related
Preferred site	Caeca	Caeca
Preferred niche	Mucus in crypts	None
Colonisation type	Persistent	Transient/intermittent
Carriage level	Relatively high	Variable
Invasiveness	Some strains	Some strains
Colonization genes	Some identified	Some identified

Although *salmonella* and *campylobacter* spp. are the predominat food-borne pathogens associated with poultry and are frequently implicated in human illness from this source, other pathogens also occur, including *Clostridium perfringens, Escherichia coli* 0157 and *Listeria monocytogenes,* together with those recognised more recently, such as *Arcobacter* and *Helicobacter* spp. This paper will consider the significance of the key organisms as meat contaminants and the extent to which their incidence on poultry products is likely to be affected by application of the Hazard Analysis Critical Control Point (HACCP) system and development of Quantitative Risk Assessment as food-safety management tools.

Salmonella* and *Campyplobacter

Contamination of poultry carcasses and parts with these organisms is well documented and data are available for many parts of the world (e.g Waldroup 1996: Simmons *et al.,* 2003), although inter-country comparisons are not usually possible, because of differences in sampling and methods of testing. Most salmonella found on poultry meat are non-host-specific and are considered capable of causing human food poisoning. The thermophilic campylobacters are mainly *C. jejuni,* which is the principal cause of human campylobacteriosis, but other so called 'Campylobacteria' also occur frequently, and includes species of *Arcobacter* and *Helicobacter pullorum*. Their potential for causing human illness has been discussed by Corry and Atabay (2001). For processed poultry, both the proportion of positive samples and the number of organisms present per unit sample is greater for C*ampylobacter* than it is for *Salmonella,* reflecting the higher level of intestinal carriage at slaughter (Table 1), which can be up to 10^9 cfu/g. With *Salmonella,* there is wide variation in the incidence of positive carcasses, but counts rarely exceed 200cfu/carcass, well below level normally associated with food poisoning. However, both types of bacteria include strains that are invasive in poultry and can penetrate internal organs or deep tissues of the bird, where the organisms may be less readily destroyed by cooking. On the surface, *campylobacter* contamination tends to be relatively high, up to 10^6 cfu/carcass. Since the ineffective does is only a few hundred viable cells, illness can easily result from handling raw poultry without suitable hygiene precautions, and is a hazard for new staff in poultry processing plants.

Salmonella survive well in the environment, but campylobacters appear less well-adapted to survival outside the alimentary tract of warm blooded animals. Also, growth only occurs under conditions of high moisture, reduced oxygen and an environmental temperature above 30^0C. The organisms are particularly sensitive to drying and the effects of freezing and thawing, which can cause a 1-2 log reduction in the level of contamination on poultry meat. However, campylobacters have many different hosts, they colonise at high levels and therefore are shed into the environment in large numbers. There is still much debate about possible survival mechanisms outside the host, including the ability to exist in a supposedly dormant form, in which the organisms appear to be viable, but non-culturable by conventional methods. From the practical viewpoint, campylobacters can persist as contaminants of poultry products throughout the entire supply chain and remain detectable by culturable methods. A key factor in their survial may be their attachment to, or entrapment in, poultry tissues during carcass processing. In this situation, their resistance to adverse conditions, like that of other bacteria, is significantly increased. Thus, the organisms can survive on carcasses during processes such as scalding, washing and water chiling, that might otherwise remove or destroy them.

Clostridium perfringens

As a cause of human food poisoning, this is not among the more dangerous pathogens. It is , however, a spore-forming organism and some strains produces spores that are unusually heat-resistant. Therefore, unlike vegetative bacterial cells, the spores are not necessarily destroyed by normal cooking and may subsequently germinate and outgrow to hazardous levels, if post-cooking storage is inadequate. In fact, most outbreaks involve strains that produce the more heat-resistant spores. In a survey of food –poisoning outbreak associated with poultry in England and Wales during 1992 – 1999, Cl., *Perfringens* was found to be some responsible for 21% of the outbreaks, second only to Salmonella as a causative agent (Kessel *et al*., 2001). In some instances, the problem arose from consumption of contaminated turkey at Christmas time, when storage of the larger, whole carcasses used for festive meals appear to have been at fault. The organism is an obligate anaerobe that is relatively tolerant to oxygen and can be found in low numbers in the alimentary tract of poultry. When present in meat crevices etc, growth is favoured by conditions in which oxygen has been dispelled by cooking. However, since growth of the organisms cannot occur if the meat is held below 15^0C, the problem is easily avoided by refrigerated storage.

***Escherichia coli* 0157**

Verocytotoxin-producing strains of *E.coil* (VTEC),cause diarrhoea and haemorrhagic collitis in humans and can lead to potentially life-threatening sequelae such as haemolytic uraemic syndrome and thrombotic thrombocytopaenic purpura. Although VTEC strains occur in a wide range of O serogroups, the most important in human disease is 0157,which accounts for almost all major foodborne outbreaks in Europe and the USA. In England and Wales ,the first case involving this organism occurred in 1982 and reported cases have increased steadily since then ,reaching a peak of 1087 in 1997(PHLS data).While VTEC 0157 is mostly found in ruminant animal ,it is occasionally associated with other livestock and various foods of animal origin .To what extent is the organism a matter of concern in relation to poultry? An outbreak in the UK that was associated with eating turkey roll was reported by Salmon *et al* .(1989)and two further outbreaks linked to chicken dishes were mentioned by Kesse *et al.* (2000). Experience suggests that VTEC 0157 is rare in poultry ,whether in the live birds or on processed products ,and when it has been found, tests for the necessary virulence factors have not always been carried out On the other hand, strains lacking Shiga toxins genes have been isolated from patients with typical disease symptoms (Schmidt *et al*. 1999).

In a survey of retail meats in the USA, Doyle and Schoeni (1987) found in VTEC 0157 in 1.5% of 263 samples of chicken and turkey leg meat. Although Heuvelink *et al.* (1999) could find no VTEC 0157 in chicken faeces, 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 haemolysin. Because of these virulence factors, the strain was clearly capable of causing illness in man. Only turkeys had been kept on the farm in question, so transfer of the strain from other livestock was unlikely. VTEC other than 0157 were found in 12% of retail chicken samples and 7% of turkey samples in the USA by Samadpour *et al.,* (1994).

Despite the rarity of VTEC 0157 in poultry, experimental studies have shown that chicks can be readily colonized 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 (Stavic *et al.,* 1993) showed that the organism was present, following challenge , on caecal mucosa and in the content of the lumen. The extent of colonization depended on dose, age, breed and time after exposure. However, colonization could be reduced by competitive exclusion(CE) treatment, using a culture of faecal material from a pathogen free donor. Bird. Harkinen and Schneitz (1996) obtained a 4-log reduction in colonization, when a commercial CE product was used to treat chicks before challenge.

Since VTEC 0157 is capable of colonizing poultry without causing illness in the birds, is present in some wild-bird vectors, survives well in soil and is able to grow in chicken manure held at ambient temperatures, it is surprising that the organism is not found more often in commercial broiler flocks. The significance of non- 0157 VTEC, which also appear to occur infrequently in poultry, needs to be investigated.

Listeria monocytogenes

The organism is a leading cause of food-related mortality and morbidity in man, and the majority of cases are believed to be food-borne. The symptoms vary widely and those affected are frequently among the most vulnerable groups in society. Nevertheless, despite the common occurrence of *L. monocytogenes* in a variety of foods, human listeriosis is relative rare, which may be due in part to the high infective dose of 10^9 viable cells that appears to be necessary in most cases (Smerdon *et al.,* 2001). The organism is common on raw poultry meat and has been found on chicken, turkey, duck and pheasant. Numerous surveys have shown that more than 50% of processed chicken are likely to be positive, although numbers are usually low, even < $1/cm^2$ of skin.

The health hazard from contaminated, raw poultry is mainly one of cross-contamination in the chicken, where the organism may spread to cooked foods or other ready-to-eat items, such as salad vegetables. There is also a potential problem with cooked poultry produced commercially. Although normal cooking destroys listerias, recontamination can occur during post-cooking handling at the factory, even with the most rigorous hygiene control. Since pre-cooked items are not necessarily reheated by consumers before being eaten, and the organism is capable of growth under chill conditions, strict microbiological limit values are considered necessary. At one extreme , in the USA, there is zero tolerance for *L. monocytogenes* in ready-to-eat poultry products, and periodical recalls of contaminated product batches have cost many millions of US dollars. A different approach is taken in the UK, and counts of *Listeria* spp. below 20 cfu/g are considered 'satisfactory'. In a recent survey of barbecued chicken samples at retail (Williams *et al.,* 2002), all 221 samples examined were in this category. Such a low level of product contamination does not suggest that any significant growth of the organism had occurred in positive samples.

Control of product contamination

For food to be entirely safe from the microbiological viewpoint, it would need to be free from all the pathogenic organisms. It is widely recognized, however, that this is not a realistic goal for raw poultry meat. There is still no economically viable means of eliminating foodborne pathogens in poultry-meat production, without the use of ionising radiation, which is presently unacceptable to most consumers. Therefore, some level of product contamination must be tolerated, although this varies widely from one country to another, especially in relation to *Salmonella.* In Sweden, which has a small poultry industry, the prevalence of *Salmonella* contaminated poultry meat has been less than 1% for many years and the organism are rarely found in retail samples due to rigorous surveillance and control programmes that are relatively costly to operate (Persson and Jendteg, 1992). Food from which salmonellas are isolated in Sweden is, by law, considered unfit for human consumption. By contrast, countries with larger, more complex poultry industries find control of *Salmonella* more difficult and subject to cost constraints. In the UK, improved practices in production and processing have led to a steady decline in the contamination rate, the latest survey of retail chicken showing only 5.7% of samples positive, in comparison with almost 80% some 20 year ago (Report 1996). This can be attributed largely to control at farm level, especially in relation to *S. enteritidis* (Table2). Recent data for the USA (Simmons *et al.,* 2003) showed 33.9% of whole carcasses positive over a 20 – week sampling period. In the USA and many other countries, detection of *Salmonella* in a particular lot of poultry does not imply that the lot should be condemned for that reason, bearing in mind that the small number of cells usually present on a contaminated item is unlikely to be direct cause of human illness. Also, regular rejection of contamination lots would be economically unaccepted on the scale required. Instead, there is growing emphasis on the application of preventive measures within the Industry and there is now much reliance on the HACCP system for controlling foodborne pathogens in poultry processing.

Table 2 Changes in Incidents of some *Salmonella* serotype in British Chickens.

Incident (%) Serotype	1997	1998	1999	2000	2001	2002
Enteritisis	21.0	16.6	3.2	0.9	0.8	1.3
Typhimurium	5.8	7.5	6.7	3.5	6.1	4.1
Senftenberg	5.6	11.4	12.4	21.6	16.7	12.3
Livingstone	1.9	3.6	6.3	4.0	8.9	14.0
Liverpool	5.9	1.6	2.1	2.6	6.9	3.6
Mbandaka	10.2	6.2	9.2	3.5	6.6	5.9
Thompson	6.2	5.3	5.3	6.2	6.5	3.6

(Date: *veterinary laboratories Agency, Weybridge, UK)*

The microbiological hazards in the processing operation are well known and are often difficult to control effectively, because of the technological limitations in the process that can lead cross-contamination of the carcasses being processed. Implementation of the HACCP system does not overcome this drawback, but has a number of clear benefits, including the following:

1. The system ensures regular monitoring of the process as a whole.

2. Hygiene control is optimized, within the above-mentioned constraints, thereby providing evidence of 'due diligence' on the part of the processor, as required by UK food law.

3. Checking of control parameters and recording of results are in integral part of the system.

4. Compliance with hygiene legislation is ensured

5. Staff awareness of food-safety requirements is increased.

6. As a result of national HACCP implementation, operational standards

across the industry become more uniform.

Cross-contamination of carcasses can occur at virtually every stage of the process and currently there is little evidence that this problem is significantly reduced by the application of HACCP principles, without a decontamination step . also uncelar is the effect of the HACCP system on levels of carcass contamination, although this will vary according to the type of process used and permitted intervention measures in different countries. The most effective type of process for reducing contamination is likely to be one in which carcasses are immersion-chilled in chlorinated water and then frozen. In USA, where water-immersion chilling is the norm and super-chlorination of process water is permitted, there is also the option to use a chemical decontamination treatment for carcasses, which may involve substances such as trisodium phosphate, acidified sodium chlorite or peroxyacetic acid (Russell, 2003). In this respect, there is currently a very different situation in the EU, because super-chlorination is not allowed, immersion chilling has been largely replaced by air chilling or evaporative cooling, and any form of chemical decontamination is unacceptable. Therefore, in the case of fresh carcasses that are air chilled, there is no progressive reduction in carcass contamination (Allen *et al.,* 2000; Fluckey *et al.,* 2003). Moreover, there is no Critical Control Point at which a significant reduction in pathogen contamination can be guaranteed. However, this unsatisfactory situation may change in 2006 (Report, 2003). Without the use of processing aids to improve hygiene, the greatest reduction in carcass contamination are likely to come from technological developments in the process that are designed to improve hygiene, as long as these are acceptable to the industry. For example, a process for simultaneous scalding and plucking of broilers, although not adopted commercially reduced levels of Enterobacteriaceae on carcasses by one hundred- fold in experimental trials (Mulder, 1985). On the other hand, a study aimed are reducing *Campulobacter* contamination by merely optimizing existing processing procedures, achieved much smaller improvements (Mead *et al.,* 1995). Possible benefits from physical carcass decontamination treatments that are being developed to reduce levels of *Campylobacter* are shown in Table 3.

Table3 Effects of physical decontamination treatments in reducing levels of *campylobacter*

Treatment	*Log_{10} reduction
Cooling/drying, 20^0C/(C)	0.3
Drying/heating:	
30^0C, 15 min (S)	1.0 –2.0
40^0C, 15Min (S)	2.0 – 3.5
Crust – freezing (C)	0.4
Steam at 100^0C, 12 sec(C)	2,5

* Carcasses (C) or skin portion (S) inoculated with a poultry strain of *C. jejuni (*Corry *et al.,*2003 and personal communication

Mandatory use of the HACCP system in US processing plants, which began in 1997, is coupled with performance standards that include a *Salmonella* prevalence of 20% for post-chill broiler carcasses (Federal Register, 1996). How cost-effective has this approach been in reducing human samonellosis? In posing the question, it must be acknowledged that the *Samonella* status of processed carcasses depends ultimately on control measures taken on the farm, which are not addressed directly in the legislation. Attempt to meet the requirements of the so-called 'Mega-Reg' have involved a 30-40% increase in the use of clean water during processing, and overall costs are said to be several times higher than official forecast (Ollinger and Mueller, 2003). So far, there is no real evidence that human salmonellosis has fallen in USA as a result of HACCP implementation. In the year 1999, there were 32 782 reported isolations of *Salmonella* from human cases, increasing to 33 310 in 2000 and then decreasing to 31 675 in 2001 (CDC data). Thus, the recent situation has been relatively static and it could be that the performance standard of 20% is not yet low enough to impact on human salmonellosis.

Microbiological risk assessment (MRA)

MRA is a developing concept, which is complementary to the application of HACCP principles. As defined by the Codex Alimentarius Commission (CAC, 1999), it includes hazards identification, exposure assessment, hazard characterisation. The concept is discussed in relation to poultry by Kelly *et al. 2003).* It is important not only in quantifying the risk of human illness from a pathogen or microbial toxin associated with poultry, but in determining the extent to which the risk can be reduced by specific intervention measures. Thus, the effect of controlling the hazard at a particular Critical Control Point can be quantified with this approach.

Quantitative risk assessment vary in mathematical complexity, depending on the question being asked. Often, they require a diversity of data that is sufficient to account for any variation that occurs. In practice, data sets are usually far from complete and may be subject to considerable uncertainty. This problem is compounded by the dynamic nature of microbial populations, which undergo continuous change. Dealing with uncertainty has been a feature of the development of MRA and is clearly evident in the case of *Campylobacter* infections associated with chicken consumption. Here, the true extent to which human cases are derived from eating chickens is unknown, it has to be assumed that all strains of the organism have the same potential to cause human illness and that their pathogenic and survival properties are identical. Also , there is a general lack of data on level of product contamination at different stages of the supply chain and during subsequent handling prior to contamination. Nevertheless, the MRA described by Kelly *et al.,* (2003) makes some important predictions and highlights the effect of freezing poultry meat, which, more than other mitigation strategic examined, will reduce both the chance and level of subsequent human exposure.

Increasingly, risk assessment is being used as a scientific tool to evaluate human health risks from hazardous agents present in foods. In this respect, Munday *et al* (2003) have identified 36 risk assessments on *Salmonealla,* 18 on *Campylobacter* and 16 on *Listeria,* including completed and on-going studies in both developed and developing countries, as well as those undertaken by FAO/WHO on *Salmonealla* and *Campylobacter* in broilers. However, it is necessary to recognize that MRA is still in its infancy and the degree of uncertainty is high, indicating that much remains to be done to fill the data gaps and refine the mathematical methods involved. Ultimately, MRA will ensure that public health policies have a sound scientific basis and will be directed towards the most effective control strategies.

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