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## **Campylobacter in Poultry: Incidences and Possible Control Measures**

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### **ABSTRACT**

Campylobacters are Gram-negative, nonspore-forming, curved spiral or rod shaped and microaerophilic in nature. They are also oxidase and catalase positive and are unable to grow at 25°C under aerobic condition. In recent years, campylobacters have been implicated in most foodborne outbreaks and are considered important human pathogen. They are known to cause enteritis, bacteremia, endocarditis and periodontal diseases in humans and animals, and their infection can lead to chronic sequelae such as Reiter syndrome and Guillain-Barré syndrome in humans. Poultry have been identified as a major reservoir for campylobacters. Cross contamination of campylobacters from contaminated live birds to carcasses, poultry products, the environments, other products and animals species is eminent. Nevertheless, poultry meat and products are still preferred by most people and are consumed worldwide without much traditional or religious restriction. Furthermore poultry meat is considered healthier, due to their lower fat content compared to ruminants. Other sources of campylobacters such as wild birds, rabbits, birds, insects, sheep, horses, cows, pigs, domestic pets, vegetables, shellfish and water have also been recognised. Consumer awareness for food safety is increasing and consequently the demand for poultry meats that are free from pathogenic organisms. A discussion on campylobacter and its association with poultry is important to create more awareness on need to reduce campylobacter colonisation in poultry, transmission, cross contaminations and infections.

**Key words:** Campylobacters, cross contamination, food safety, infection, poultry

### **INTRODUCTION**

Campylobacters are very important cause of foodborne human diseases. Campylobacteriosis (campylobacter infection), have been describe as an emerging foodborne disease (Houf and Stephan, 2007) and they are now said to be the major cause of bacterial gastroenteritis in humans (Kwan *et al.*, 2008). In addition they have now been estimated to be the most common causative agent of foodborne illnesses, followed by non-typhoidal *Salmonella* and *Shigella* spp. (Mead *et al.*, 1999). For these reasons they are among the most studied groups of bacteria.

It has been estimated that approximately one percent of the population in Western Europe is infected each year (Humphrey *et al.*, 2008), this equates to about 600,000 cases in UK. In the United States, the number of human campylobacteriosis cases per year is estimated to be around 2.1 to 2.5 million and 2,000 deaths are attributable to the infection (Altekruse *et al.*, 1999). Such reliable data are not available in developing countries; although, Taylor and Blaser (1991) and Koulla-Shiro *et al.* (1995) reported on the isolation of campylobacter in humans to range from 5 to 20% in developing parts of Asia, Africa and Latin America, in surveys of children with diarrhoea.

In addition, (Reinthaler *et al.*, 1998) observed that *C. jejuni* was the leading cause of diarrhoea among 322 travellers returning from Asia, Africa and Latin America to Australia.

Poultry have been reported by several authors to be the leading reservoir for campylobacters and thus poultry meat and products are implicated as the leading source of human campylobacteriosis (Moore *et al.*, 2005). Despite this, poultry meat and meat products are consumed worldwide. In South-East Iran Mohammad *et al.* (2006) said that the consumption of poultry products is exceedingly. Increase consumption of poultry and poultry meats products might have been facilitated by its lower fat content compared to ruminants, the use of chicken to prepare various ready-to-eat meals and the development of several poultry meat products. For instance several authors (Huda *et al.*, 2008, 2009a, b, 2010) have prepared chicken nuggets, chicken meat balls and chicken sausages from poultry meat. *Campylobacter* spp. normally colonize the gastrointestinal tract of poultry and are transferred to poultry carcasses and the environment under handling and slaughtering conditions. Other important contaminated sources such as untreated water, raw milk, cattle and food handler contamination have also been reported (EFSA, 2005; Arun, 2008).

Food safety continues to be an increasing concern to consumers and campylobacter infection in particular has emerged as an important public health problem in most areas of the world (EFSA, 2005). This makes efficient methods for the isolation and identification of *Campylobacter* species essential to facilitate clinical and epidemiological studies. This review briefly discusses campylobacters, incidences, isolation techniques and possible practices to reduce campylobacter colonization, contaminations and/or infections in poultry. The use of poultry in this review refers to domestic fowls and/or chicken.

## **CAMPYLOBACTERS AND THEIR INFECTION**

Campylobacters are small Gram-negative, nonspore-forming, curved spiral or rod shaped bacteria that are microaerophilic in nature (Corry *et al.*, 2003; Halablab *et al.*, 2008). They are catalase positive, oxidase positive and unable to grow aerobically at 25°C. They are also motile, with either uni- or bi-polar flagella, 0.2-0.5 µm wide and 0.5-8 µm long (Corry *et al.*, 2003; Moore *et al.*, 2005). The uni-polar flagellum gives campylobacter a characteristic cork-screw motility (Corry *et al.*, 2003; Song *et al.*, 2004). Furthermore, campylobacters cannot ferment or oxidise carbohydrates, but obtain their energy from amino acids or intermediates originating from tricarboxylic acid cycle (Vandamme, 2000; EFSA, 2005). This is because they lack the enzyme, 6-phosphofructokinase, involved in energy metabolism (Velayudhan and Kelly, 2002).

There are 17 species within the genus campylobacter, which can be divided into more than 60 penner serotypes (heat-stable antigens) and more than 100 Lior serotypes (heat-labile antigens) (On, 2001; De Zoete *et al.*, 2007). Two thermophilic campylobacters, *C. jejuni* and *C. coli* are the most important species considered in terms of food safety. Other campylobacter species are *C. lari*, *C. upsaliensis*, *C. fetus* (are thermophilics) and *C. concisus*, *C. curvus*, *C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. showae*, *C. sputorum* and *C. rectus* (are non-thermophilics) (On, 2001; Corry *et al.*, 2003).

Of the foodborne illnesses associated with campylobacters, *C. jejuni* is responsible for approximately 90% of all sporadic cases and most of the rest by *C. coli* (EFSA, 2005). *Campylobacter jejuni* infections have been linked to sequelae infections like Guillain-Barré Syndrome (GBS) and Miller-Fisher syndrome (Ang *et al.*, 2001); reactive arthritis and Reiter's Syndrome (characterised by arthritis, urethritis and conjunctivitis) (Bereswill and Kist, 2003) and other extra intestinal diseases affecting the neuromuscular system, for example, meningitis as well as those affecting the skin, gall bladder, pancreas, kidney, appendix, liver, blood and the bone especially in immunocompromised patients (Monselise *et al.*, 2004). A more recent study has

suggested that *C. jejuni* infections can also lead to inflammatory bowel diseases such as Crohn's Disease (Lamhonwah *et al.*, 2005). Consumption of 500 cells or less have been reported to be enough to cause mild illnesses such as diarrhoea, vomiting, headache fever, nausea, abdominal pain and muscle pain in humans (EFSA, 2005).

### INCIDENCES OF CAMPYLOBACTER IN POULTRY MEAT, PRODUCTS AND THE PROCESSING ENVIRONMENT

The main source of campylobacter infection in humans is considered to be due to the consumption or contact with undercooked poultry meat (Nauta and Havelaar, 2005), cross-contamination from raw poultry meats and products to foods that are consumed without further heating (Studahl and Andersson, 2000). Poultry is considered as a probable source and/or vehicle for transmission because similar serotypes and phage types have been isolated from both poultry and humans with gastroenteritis (Saito *et al.*, 2005). A summary of the incidences of campylobacter in poultry processing plants, meat and products is found in Table 1. From Table 1,

Table 1: Incidences of campylobacter in poultry processing plants, meat and products

Samples	Incidences (%)	Reference
Chicken meat	64.70	Sallam (2007)
Breast	64.40	
Thighs	70.00	
Wings	77.10	
Livers	65.00	
Gizzards	45.00	
Hearts	40.00	
Breasts	62.10	Suzuki and Yamamoto (2009)
Thighs	58.70	
Wings	62.30	
Fillets	23.70	
Gizzards	62.20	
Livers	62.30	
Hearts	33.30	
Imported frozen chicken from Brazil	28.30	
Imported frozen chicken from China	9.50	
Imported frozen chicken from Thailand	55.00	
Imported frozen chicken from USA	5.30	
Imported frozen chicken from Malaysia	0.00	
Abattoir	54.00	Figueroa <i>et al.</i> (2009)
After defeathering (plant A and B)	15.00 and 46.00	
After evisceration (plant A and B)	37.00 and 61.00	
After chilling (plant A and B)	23.00 and 46.00	
Retail chicken products	Greater than 71.00	Saito <i>et al.</i> (2005)
Poultry flocks	41.10	Atanassova and Ring (1999)
Broiler carcasses	45.90	
Slaughtered broilers	45.90	Bryan and Doyle (1995)
Poultry meat	41.00	
Chicken meat	70.70	Zhao <i>et al.</i> (2001)
Broiler carcass	71.90	Ghafir <i>et al.</i> (2007)
Broiler fillets	82.30	
Broiler liver	68.70	
Layer carcasses	86.60	

the percentage incidences of campylobacter in the various samples differ from each other. Layer carcasses showed the highest incident (86.60%) level. This might be due to the longer period in which layers are raised in layer houses which may be harbouring campylobacters or poor processing and handling practises. Higher campylobacter incidences were reported by Ghafir *et al.* (2007) in their samples. Imported frozen chickens had the lowest incidences and in Malaysia campylobacters were not isolated from the frozen chickens. Freezing can reduce the number of campylobacters on a product (Adzitey, 2008). Campylobacters were also isolated from the abattoir and thus, cross contaminations of successive flocks is possible at the abattoir. Within the plants after evisceration showed the highest campylobacter incidence signifying that the evisceration area in a plant is a critical control point. With the chicken parts it appears wings are more easily contaminated. The isolation of campylobacters from chicken carcasses also confirms the fact that people who consume chicken are at a risk of campylobacteriosis.

### **ISOLATING AND DETECTING OF CAMPYLOBACTERS IN POULTRY**

Efficient and reliable techniques for the isolation and identification of *Campylobacter* species in poultry are essential to facilitate clinical and epidemiological studies. The use of the conventional method for detecting and isolating campylobacters has been mostly relied on. The conventional method involves enrichments and/or plating onto selective media and biochemical confirmation (Corry *et al.*, 2003). Enrichments broths used for isolating campylobacters include Cefaperazone Amphotericin Teicoplanin (CAT), Hunt and Radle, Bolton, Exeter, Hunt, Preston, Park-Sanders, Doyle and Roman, Rosef, blood-free enrichment and Campylobacter enrichment broths. While plating has been achieved on modified cefoperazone charcoal deoxycholate (mCCDA), Columbia blood (CBA), Campy-Cefex, CAT, blood, Karmali, Abeyta-Hunt, Blaser and Skirrow agars. Biochemical tests carried out for campylobacters also includes oxidase, catalase and glucose utilization. Incubation is done between 25 to 42°C under microaerobic (5% oxygen, 10% carbon dioxide and 85% nitrogen) condition. Thermophilic campylobacters cannot grow below 32°C (Corry *et al.*, 2003) but grows optimally at 42°C which is nearer the body temperature of birds. This perhaps favours the growth of thermophilic campylobacters (Horrocks *et al.*, 2009). The pH range at which campylobacters grow well is between 5.5 to 8.0, although, the pH of many isolation media is not specified but normally it is near neutrality (Corry *et al.*, 2003). More details of the methods for isolating and detecting *Campylobacter* species have been described by Hunt *et al.* (1998) and ISO (2004).

Conventional methods for the detection and isolation of *Campylobacter* species are said to be relatively slow, laborious and less efficient (Keramas *et al.*, 2004). As such, various rapid methods categorised broadly into immunological (e.g., latex agglutination test, ELISA), nucleic acid (e.g., Polymerase Chain Reaction (PCR) based methods) and growth-based methods have been applied. With thermophilic campylobacters, flagellin typing (FlaA/FlaB), Pulsed Field Gel Electrophoresis (PFGE) and Amplified Fragment Length Polymorphism (AFLP) are commonly employed to identify and compare distinct genotypes among humans and animals. These methods determine specific thermophilic *Campylobacter* strains based on precise identification of genomic DNA. Nevertheless, conventional methods are widely used and have the advantage that they are cheaper, detect only viable campylobacters and also yield isolates that can be studied and further characterised (Engberg *et al.*, 2000; Corry *et al.*, 2003).

## **COLONIZATION AND TRANSMISSION OF CAMPYLOBACTERS BY POULTRY**

Usually campylobacters colonize the gastrointestinal tract of poultry. It has been noted that once campylobacter is established within an individual bird, horizontal transmission often occurs rapidly through the flock (Horrocks *et al.*, 2009). A number of factors also contribute to risk of colonization and spread of campylobacters. They include flock size, environmental water supplies, insects, rodents, airborne isolates, another house on-farm, on-farm staff, other animals on farm and depopulation event (Adkin *et al.*, 2006; Horrocks *et al.*, 2009).

Campylobacters can infect chickens at a much younger age and defaecation will spread the pathogens among the entire flock (De Zoete *et al.*, 2007). Herman *et al.* (2003) examined day old chicks from hatcheries prior to rearing and found that they were campylobacter negative. El-Shibiny *et al.* (2005) isolated campylobacter from chickens as young as 8 days old which were kept on free range, although, Bull *et al.* (2006) reported that it takes averagely several weeks for a flock to be colonized. There is also some evidence that chicks are seldom colonised by campylobacters under normal commercial conditions before two weeks of age (Moore *et al.*, 2005) due to maternal antibody protection, but once infected the birds will remain infected for life (Gibbens *et al.*, 2001). Subsequently, the number of colony forming units (cfu) necessary to initialize colonization within birds may play a key role in horizontal transmission (Horrocks *et al.*, 2009).

Other studies have suggested that aerosol and vertical transmission of campylobacter is possible, which is opened to debate (Berndtson *et al.*, 1996; Petersen *et al.*, 2001). There is the controversy of whether campylobacters are transmitted by aerosols or not since campylobacters have been isolated from aerosols in campylobacter positive flocks (Berndtson *et al.*, 1996). Conversely, the same researchers reported that campylobacters were not isolated from aerosols of campylobacter negative flocks. A study by Sahin *et al.* (2003) suggested that *C. jejuni* has the potential to enter eggshells under specific conditions. *Campylobacter jejuni* has been recovered from the reproductive tracts of healthy laying and broiler breeder hens (Camarda *et al.*, 2000; Hiett *et al.*, 2002) and from the semen of commercial broiler breeder cockerels (Cox *et al.*, 2002). However, Bull *et al.* (2006) were unable to confirm vertical transmission from parents to their progeny in their work.

Campylobacter positive flocks are also influence by geographical region and season. Higher percentages of Campylobacter positive flocks and infections have been reported in the summer than the winter (EFSA, 2005). Louis *et al.* (2005) also found that increased campylobacter rates were correlated with temperature. Campylobacters may survive better in temperate regions compared to tropical regions due to the low oxygen tension in temperate regions during some part of the year.

At poultry processing plants, campylobacters are normally found on the skin of the carcass due to contamination from the gastrointestinal contents. Transportation conditions from farms to the abattoirs also increases cross contamination among birds. Slaughtering, dressing and further processing are the potential sources for the spread of *Campylobacter* species from the gut contents onto carcasses. Different flocks are processed one after the other on the same processing line (within a period) and undergo scalding, plucking and evisceration, all of which are opportune times for campylobacter dissemination (Moore *et al.*, 2005). Despite the role poultry plays in the spread of campylobacters, it has been reported that they appear harmless in poultry but they live as commensals to each other (Verwoerd, 2000).

## **MEASURES TO REDUCE CAMPYLOBACTERS IN POULTRY**

Measures to reduce campylobacters in poultry will rely heavily on careful management practices to reduce colonisation, transmission and cross contaminations. At the farm it will involved the

Table 2: Strategies to reduce campylobacters in poultry

Strategy	References
Antimicrobial treatments	Horrocks <i>et al.</i> (2009)
Administered of bacteriocins	Svetoch and Stern (2010)
Administration of bacteriophage	Wagenaar <i>et al.</i> (2005)
Chemical feed additives	Horrocks <i>et al.</i> (2009)
Nitrocompounds (2-nitro-1-propanol)	Horrocks <i>et al.</i> (2007)
Deaminase inhibitors (diphenyliodonium chloride and thymol)	Anderson <i>et al.</i> (2007)
Competitive exclusion	Hakkinen and Schneitz (1999)
Feed supplementation using organic acid	Heres <i>et al.</i> (2004)
Carcass treatment with 1 ppm of free chlorine	Blaser <i>et al.</i> (1986)
Addition of chlorine to drinking water	Pearson <i>et al.</i> (1993)
Treating of carcass with lactic acid or alkaline chemicals	Okolocha and Ellerbroek (2005)
Vaccination of chickens	De Zoete <i>et al.</i> (2007)
Cetylpyridinium chloride (between 0.1 to 0.5 %)	Waldroup <i>et al.</i> (2010)
Freezing and thawing	Adzitey (2008)
Inside-outside bird washers	Smith <i>et al.</i> (2005)

adherence to strict hygiene and biosecurity practices. During transport a period of starvation will reduce the shedding of faeces and consequently the spread of campylobacters. Transportation crates should be well disinfection and overcrowding in crates should be avoided. Slaughter of uninfected flocks prior to infected flocks and by careful attention to major points of cross-contamination on the line will all help to reduce contamination. Corry and Atabay (2001) reported that a more effective measure to reduce campylobacter contamination would be to use a terminal decontamination step, such as trisodium phosphate, lactic acid, atmospheric steam or gamma irradiation. Table 2 further summarizes the strategies that have been suggested to be employed to reduce and/or control campylobacter in poultry.

Wagenaar *et al.* (2005) showed that the administration of bacteriophage significantly reduced *C. jejuni* concentrations in broilers. Certain nitrocompounds inhibit the oxidation of formate and hydrogens, both of which are important reducing substrates used by campylobacters for energy conservation during respiration (Borden, 2004; Smith *et al.*, 1999; Horrocks *et al.*, 2007). The use of these compounds will therefore reduce the ability of campylobacters to conserve energy for respiration and this may help reduce their numbers. Such compounds can also be used in feeds as additives (Horrocks *et al.*, 2009). Competitive exclusion is the administration of mixed cultures orally to increase resistance to infection. Although, this has been used with little success; Horrocks *et al.* (2009) showed that, it has been used mainly in neonates to prevent colonization of undesirable microflora and may be less effective in displacing established species. Supplementation of feeds with some selected organic acids reduced campylobacter concentrations in faecal samples of broiler chickens but had effect on growth (Heres *et al.*, 2004). The organic acids might have reduced feed palatability and subsequently feed intake in birds. Meanwhile, vaccines for campylobacter appears to be unavailable although, De Zoete *et al.* (2007) were of the view that the rapid development of knowledge in the biology of campylobacter, field of molecular vaccinology and immunology provides the required setting for the development of an effective vaccine against *Campylobacter* in poultry. Waldroup *et al.* (2010) reported that between 0.1 to 0.5% cetylpyridinium chloride appears to be the most efficacious antimicrobial treatment available for controlling *Campylobacter* on poultry carcasses. The use of chlorine and lactic acid to reduce campylobacter is also supported by Blaser *et al.* (1986) and Pearson *et al.* (1993). When, Smith *et al.* (2005) used

inside-outside bird washers they found a reduction in the incidence of *Campylobacter* from 22/36 positive carcasses to 1/36 positives. Adzitey (2008) found that freezing (-80°C) and thawing (at room temperature) of poultry skin (inoculated with *C. jejuni* and *C. coli*) thrice was enough to kill all campylobacters.

## CONCLUSION

Campylobacters are very important foodborne pathogen that continues to catch the attention of researchers, food processors, consumers and all stakeholders. *Campylobacter* species infection has emerged as a leading foodborne illness, surpassing salmonellosis. Their infections can result into life threatening disorders like Reiter syndrome, Guillain-Barré syndrome and Crohn's Disease. Although reservoirs for campylobacters exist in different sources, poultry are considered the major and most common source. Efficient isolation and detection techniques are important in the surveillance of campylobacters and their infections. Establishing of proper control and management strategies from the farm through to the consumer is essential to reduce the incidence of campylobacteriosis.

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