Campylobacter update – the challenge

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Campylobacter is now recognised as the leading cause of zoonotic enteric infections in most developed and developing countries (WHO, 2001), with more than 90% of human cases attributed to Campylobacter jejuni.

In many developed countries, the reported incidence has increased steadily over the last 20 years, often exceeding that of salmonellosis, but, as with other agents of enteric disease, there is under reporting, and the true incidence is likely to be considerably higher.

In the USA, it has been estimated that, until recently, there were 2.4 million cases per year, affecting nearly 1% of the entire population. The infections resulted in an estimated 13,000 hospitalisations and 124 deaths. A recent decline in reported cases has been associated with new control measures in the US poultry industry, but it remains to be seen whether the reduction can be sustained.

Guillain-Barré Syndrome

While the predominant symptom of the disease is profuse diarrhoea, there are also relatively rare, but severe sequelae, including Guillain-Barré Syndrome (GBS), which is a paralytic condition, reactive arthritis and even septicaemia in immunocompromised individuals, such as AIDS patients. Cases of GBS from campylobacter infection can require intensive hospital treatment and lead to long term disability. Now that polio-myelitis has been eradicated in the western world, GBS appears to be the most common cause of acute, flaccid paralysis.

There is also a considerable impact of campylobacter infections in the developing countries, where many individuals have a poorer health status and living conditions. The incidence of infection is particularly high in children under two years of age, and campylobacter is a significant cause of malnutrition, morbidity and mortality. The disease is usually transmitted via polluted water, contact with live animals and through various environmental sources. Thus, the situation is different from that in developed regions, where contamination of food plays a larger part.

However, since most cases are sporadic, that is they not apparently linked to any others, and outbreaks are uncommon, microbiological evidence that is needed to identify specific sources or vehicles is usually difficult to obtain. Instead, useful information has come from a number of case control studies on sporadic cases and most have identified poultry products as major risk factors.

This and other evidence suggests that handling and consumption of poultry meat are the primary sources of human infection.

Further risk factors include consumption of meats other than poultry at restaurants and barbecues, drinking contaminated water or raw milk, contact with animals, whether companion or farm animals, and foreign travel.

In practice, it is difficult to establish the proportion of cases that are attributable to a particular source/vehicle and, where foods are concerned, the situation is complicated by the influence of hygiene practices in the kitchen that cannot be properly assessed in retrospect. The existence of acquired immunity in some individuals must also be taken into account.

Another public health concern associated with campylobacter is the increasing incidence of strains that are resistant to antimicrobials used in human medicine. The emergence of resistance is particularly marked in relation to the fluoroquinolone group of antibiotics, which are often needed in the treatment of severe cases of gastrointestinal disease in man. The problem is clearly related to the widespread use of fluoroquinolones in both human and veterinary medicine.

There is also evidence that their use in food producing animals, especially poultry, promotes the development of resistant strains that can infect humans via the food chain. Apart from its public health significance, the rapid emergence of such resistance is an indicator of the adaptability of campylobacter to adverse conditions.

The faecal route

The intestinal tract of birds and mammals is a major reservoir of thermophilic campylobacters, which are mostly carried asymptomatically. The organisms may enter the general environment through contaminated water, faecal transmission or via infected humans or pets.

Once outside the host, however, the survival capabilities of campylobacters appear to be dramatically reduced and, although environmental transmission is likely to play a major part in the infection of domestic livestock, there is still much debate about the behaviour of campylobacters in the environment and their means of survival.

The organisms are generally regarded as being unusually fragile and they are known to be fastidious in their growth requirements. Can they compensate for these apparent disadvantages?

In most situations, growth only occurs at an environmental temperature above 30°C and in conditions of high moisture and reduced oxygen; prolonged exposure to atmospheric oxygen usually results in a marked decline in viability.

For example, Cox et al. (2001) inoculated fresh pine shavings, empty eggshells and unused paper liners from chick delivery boxes, all of which are dry materials.

Viability in streams

A rapid die off of campylobacter was observed, beginning within minutes of inoculation. In contrast, C. jejuni has remained viable in stream water held at 4°C for up to four weeks and has been readily isolated from the biofilms that can form in the water distribution systems of poultry houses.

Like other bacteria, exposure of campylobacters to adverse conditions can cause sub-lethal injury in a proportion of the cells and these may fail to grow subsequently on selective isolation media.

Also, unfavourable conditions induce normal cells to change from their characteristic vibrioid morphology to a coccoid form, which cannot be cultured, even in the absence of selective agents.

Nevertheless, the coccoid cells retain some of the properties that are associated with continuing viability, being able to elongate in response to nutrients, when an inhibitor of DNA synthesis is present, and remaining capable of reducing certain tetrazolium salts.

Therefore, such cells have been regarded as viable, but non-culturable

(VNC). Various experiments have been carried out to determine whether VNC cells can still infect animals, but the results obtained have been conflicting.

In general, the precautions taken in designing the experiments have rarely been sufficient to preclude the presence of small numbers of normal or merely injured cells, and Boucher et al. (1994) were inclined to the view that VNC cells are a degenerative form of the organism, rather than being truly dormant.

More recently, Talibart et al. (2000) resuscitated VNC cells by injecting them into fertilised chicken eggs, thus extending the debate about the possible significance of VNC cells in the environmental transmission of campylobacters.

The fragility of the organisms is also relevant to their role in foodborne human illness and other properties have been studied in relation to survival and/or growth of campylobacters in foods and in food-processing environments.

In comparison with other types of bacteria, C. jejuni is relatively sensitive to heating, freezing and frozen storage, and to both gamma and ultraviolet radiation. Growth is inhibited by 2% sodium chloride. While being unable to multiply under normal environmental conditions, there is some evidence of aerobic growth, especially on meats, which have catalase and superoxide dismutase activities.

Whatever adaptive mechanisms campylobacters may have for environmental survival, they make up for their apparent fragility and restricted ability to multiply outside the host by being carried in the alimentary tract in relatively high numbers, by showing considerable host diversity and having a low infective dose (approximately 10 cells).

Animal reservoirs

The diversity of animals in which campylobacters are found is a major reason for the difficulty in tracing sources and vehicles of human campylobacter infection.

Although the organisms are sometimes responsible for causing diseases in animals, they are not important veterinary pathogens and, from the public health viewpoint, the main problem is symptomless carriage, which leads to contamination of carcases.

In fact, C. jejuni or C. coli are associated with all the principal meat animals: cattle, pigs, poultry, sheep, as well as a variety of other animals, both wild and domesticated, including horses, rabbits, rodents, wild birds and insects.

Companion animals, such as dogs and cats, can also be carriers of strains that infect humans. Among meat animals, only pigs seem unlikely to play any significant role in human campylobacteriosis. This is because C. coli rather than C. jejuni is the predominant species and most pig isolates belong to serotypes that are uncommon in human infections.

The degree of domestication and the conditions under which animals are reared appear to influence campylobacter colonisation. For example, outdoor flocks with access to rivers or streams tend to have a greater incidence of positive animals than those drinking only mains water. Similarly, poultry reared outdoors, as in organic or free range production, are more frequently colonised than birds kept in houses.

Among wild birds, the Galliformes are often carriers of campylobacter and so, too, are crows, magpies, gulls and domestic pigeons. Wild birds and flies are more often carriers when associated with pig and poultry farms, and here it is likely that two way transmission of campylobacters occurs.

Among domestic livestock, susceptibility to infection varies with diet, use of antimicrobials and other factors. With poultry in particular, a contributory factor is the changing nature of the bird itself, due to many years of selective breeding for faster growth rates and better feed conversion.

Although the modern broiler grows much faster than its counterpart of 30 years ago, the bird has less genetic diversity now and a reduced ability to combat disease and resist colonisation by enteric pathogens, such as campylobacter.

A persistent coloniser

In comparison with salmonella, the other major enteric pathogen associated with poultry, campylobacter is a more persistent coloniser of the alimentary tract and tends to colonise the caeca at higher levels. The organism occupies a specific niche, being present in the mucus layer in the crypts of villi, but without any obvious association with mucosal epithelium.

The typical corkscrew morphology of the cell and its polar flagellum appear to facilitate penetration of the viscous environment of the mucus. In addition, the cells are chemo-attracted to mucin, a major component of mucus, and are chemotactic towards the L-fucose moiety. Mucin itself may be utilised as an energy source for growth. Optimum levels of colonisation are dependent on the possession of intact, functional flagella and hence motility, as well as the type of flagellin expressed (flaA).

Other factors may also be involved. Some of the genes controlling colonisation have been identified. These include virulence genes and others controlling certain stress responses, so that mutants lacking the genes in question fail to colonise chicks.

The involvement of stress response genes is interesting, because these are likely to play a part in the adaptation of environmentally acquired cells to the gut of the infected animal, where conditions are very different. Although a challenge dose of only about 30 cells per bird is sufficient to colonise chicks from the day of hatch onwards, commercial flocks are rarely colonised before two or three weeks of age. In this respect, the campylobacter situation differs from that of salmonella, where susceptibility is greatest in very young birds (Table 1).

Once campylobacters are introduced, however, they spread rapidly to virtually all birds in the flock and can do so within a few days via common feeding and drinking equipment. The birds then remain colonised at relatively high levels throughout the life of the flock (approximately 10^7cfu/g of caecal contents).

There are numerous potential sources of flock infection, including any rodents, wild birds or insects that may gain access to the house, while farm staff or visitors can introduce the organisms from the external environment, through contaminated footwear and clothing. Also, contamination from neighbouring flocks can be airborne. On the other hand, manufactured feed is unlikely to be a vehicle, because the dry condition of the feed does not favour the survival of campylobacters. The position with the birds' drinking water is more complex.

When mains water is used, campylobacters will have been eliminated by the normal treatment processes, but this is not necessarily the case with bore-hole water or any other source that is untreated. Also, there may be residual contamination in the supply pipework, when a campylobacter positive flock has been present in the house and, on removal of the birds, particular attention must be given to the cleaning and disinfection of the water distribution system.

For many years, there has been an ongoing debate about the possibility that campylobacters can be transmitted vertically from parent to progeny via the egg. Usually, this route was discounted, because C. jejuni appears unable to penetrate the eggshell membranes and, in any case, it survives poorly in egg contents. Not surprisingly, attempts to culture campylobacters from hatchery samples or newly hatched chicks have generally failed. However, there has been circumstantial evidence from strain sub-typing to suggest that a single type could be transferred from breeder parents to broilers, thus suggesting vertical transmission.

More recently, the use of molecular typing methods, which offer a means of fine discrimination between strains, have supported this contention. Isolates from breeder hens and their progeny had iden-

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tical ribotype patterns and flaA SVR DNA sequences, thereby suggesting a clonal origin for the strains.

Commonest transmisson route

Despite these findings, environmental (horizontal) transmission appears to be a more common route and there are aspects of the vertical transmission theory that have yet to be explained satisfactorily. In particular, there is the usual period of at least two weeks before flock colonisation becomes evident by conventional culture.

Does this mean that only a small number of chicks are positive initially and/or the campylobacters are dormant or sublethally injured, thus requiring an appreciable length of time to become fully active again and spread among the flock?

Until these points have been clarified, there must be some doubt about the reality of vertical transmission. Detection of campylobacters in chick fluff and eggshell samples from hatcheries, using a PCR method, did not appear to be finding viable vegetative cells. If vertical transmission is a significant route for chick colonisation, then greater emphasis will be needed on control of breeder flocks and hatcheries with regard to these organisms.

However, should the apparent delay in flock infection be genuine, why does this occur when chicks can be colonised experimentally from only a small challenge dose? A possible explanation is the presence of maternal antibodies but, perhaps a contributory factor is that, under natural conditions, the challenge is small or the cells take time to adapt to the environment of the alimentary tract.

The ability to discriminate reliably between different strains of C. jejuni and C. coli is essential to the determination of sources and routes of transmission. ■

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
Colonisation genes	Some identified	Some identified

Table 1. Features of intestinal carriage in poultry – campylobacter and salmonella compared.