ZOONOTIC DISEASES, HUMAN HEALTH AND FARM ANIMAL WELFARE

Campylobacter

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Disease in man: *Campylobacter* is the single biggest identified cause of bacterial infectious intestinal disease (IID) in people in much of the developed world and is estimated to infect 1% of the population of the EU each year. Recently the World Health Organisation declared it the most important foodborne pathogen. There were ~74000 laboratory confirmed cases in the UK in 2010 (UK Health Protection Agency; HPA). For each confirmed case there are 10 more in the community, meaning that around 700,000 people were infected in the UK in 2010. *Campylobacter* is a major problem and its control would bring about a significant improvement in public health.

The disease in people was first identified in 1977 when a selective culture medium for *Campylobacter* was developed that allowed pathology laboratories to diagnose the cause of the infection. People will have been infected before then but were undiagnosed. The strains of *Campylobacter* that infect humans have quite different growth requirements from bacteria like *Salmonella,* in that they will not grow in air and require an atmosphere enriched in CO_2. These bacteria will also appear not grow below 30°C and are unlikely to multiply on/in foods, unlike two other common food poisoning bacteria, *E. coli* and *Salmonella.*

Features of *Campylobacter* infection: Clinical features of acute *Campylobacter* infection vary from mild diarrhoea lasting 24 hours to severe illness lasting more than a week. The incubation period is typically two to five days, although can be up to 11 days before the onset of diarrhoea. A common feature with children is that the diarrhoea is often blood-stained, although this can also occur in severe infections in older age groups. Diarrhoea is usually preceded by malaise and often fever. A characteristic of *Campylobacter* infection is a persistent abdominal pain, which may mimic acute appendicitis. Other symptoms include headache, backache, aching of the limbs and nausea. However, vomiting is uncommon. It is not usual to treat *Campylobacter* infections with antibiotics although this may happen when patients are admitted to hospital.

It is estimated that over 100 people die in the UK each year as a consequence of *Campylobacter* infection. The disease is most common in the very young and in elderly people (>70 years old). In the latter group, the disease can be particularly severe and it is in these people where most deaths occur. Patients with bowel cancer and/or who are immunocompromised are particularly vulnerable and in this group septicaemia is the most common cause of death (HPA).

The *Campylobacter* infectious dose for humans is thought to be very low (<500 bacterial cells in an adult)[1]. Very few data are available from outbreaks and studies to determine the exact number of cells that will cause human infection have proved inconclusive, although examination of a bottle of bird-pecked milk, which was part of a batch implicated in an outbreak at a nursery, revealed contamination levels of fewer than 10 cells of *Campylobacter jejuni* (the main species infecting man) per 100ml[2]. It should be borne in mind that when contaminated liquids are consumed many of the bacteria will be killed by acids in the stomach and *Campylobacter* are particularly acid-sensitive. It is likely that only a few cells are needed to establish infection in the intestine.

Long-term sequelae of *Campylobacter* infection: Acute *Campylobacter* infection is quite severe enough in its own right but around 1% of cases go on to develop long-term complications. These include neurological, rheumatological and gut health problems. The neurological problems are the most severe and one, Guillain-Barré syndrome (GBS), is particularly dangerous[3]. GBS is an acute, bilateral, ascending paralysis occurring typically 1-3 weeks following onset of diarrhoea. The association with *Campylobacter* appears to be restricted to infection with *C. jejuni*, which is the most commonly reported infectious trigger for GBS. It is believed that GBS is a consequence of some surface antigens on *Campylobacter* cells having similar structures to those of nerve endings. Thus when antibodies are mounted by infected people against *C. jejuni*, they also attack their own nerve endings, damaging them. The most common sequelae of *Campylobacter* infection are long-term bowel problems such as irritable bowel syndrome (IBS)

The economic burden of *Campylobacter* infection: Apart from the public health problems caused, *Campylobacter* infection also carries a large economic burden. In the United States, the annual estimated cost is around US$4.3 billion. The average cost of a case of acute *Campylobacter* infection (excluding long-term sequelae) in England in 1995 was estimated to be £315. The figure would be much higher now and given that there are approximately 700,000 cases in the UK in 2010, the burden to the UK economy is enormous and may exceed £2 billion each year. The main burden on the economy is health care costs and lost work and school days.

Seasonality of *Campylobacter* infection: A striking feature of the epidemiology of human *Campylobacter* infection is its remarkably pronounced and consistent seasonal pattern[4]. There is a sharp rise in cases in the late spring and early summer, which levels off in June and July in the Northern Hemisphere. There is a suggestion that the precise timing of the seasonal peak varies with longitude. Studies have shown that in Europe, weekly case numbers peaked earliest in western-most countries, peaking later further east. Although well characterised, the epidemiology of the seasonal peak in humans is not well understood. Various hypotheses have been suggested, including buying puppies in the summer months, consumption of bird-pecked milk and and/or barbequed food or exposure to environmental risks. Scientists at the University of Liverpool are undertaking a large study to try and identify the cause of the summer peak in the UK. Consumption of poultry does not seem to be important in this.

Risk factors for *Campylobacter* infection: Most *Campylobacter* infections are sporadic and do not form part of outbreaks, although outbreaks caused by chicken liver pate are becoming more common (HPA data). Even so, these comprise less than 1% of the total number of cases. Risk factors for sporadic infections are usually identified by conducting case-control studies. These compare the infected with matched well controls and in so doing identify food consumption patterns and/or behaviours that increase the risk of infection. These studies have demonstrated the complexity of the epidemiology of *Campylobacter* infection. Consumption of untreated water or rainwater have been implicated as risk factors for *Campylobacter* infection as has exposure to contaminated water by activities as water-skiing and wind surfing[5]. A study in Sweden found positive associations between *Campylobacter* infection in households and average water-pipe length per house[6]. These observations suggest that drinking water might also be important factors in explaining at least part of the burden of human campylobacteriosis. In addition to risks from food (especially chicken, see below) and water consumption, contact with animals (either domestic pets or farm animals) has also been implicated in infection. Consumption of unpasteurised milk is also an important risk. Underlying medical conditions such as diabetes or reducing gastric acidity through the use of proton pump inhibitors also increase the risk of acquiring *Campylobacter* infection[7].

Chicken as a source and vehicle of human *Campylobacter* infections: Despite other vehicles or exposures being identified as risks for *Campylobacter* infections, there is no doubt that internationally most people become infected because they have eaten or handled contaminated poultry. All types of poultry can carry *Campylobacter* but the biggest risk is chicken and this meat is estimated to be responsible for up to 80% of cases of infection in the EU (EFSA). This is a major public health issue, which must be controlled. At present, the international poultry industry does not have reliable tools to protect chickens from *Campylobacter* infection but research is identifying possible measures. In the UK there is a large body of collaborative research involving poultry companies, food retailers and the academic community.

In most developed countries, the number of *Campylobacter* cases has been increasing over the last 20 years. Improved diagnosis may play some part in this, although most clinical laboratories have not significantly changed their techniques over this time period. It is difficult to escape from the conclusion that the rising tide of cases is associated with increased chicken consumption. If the UK is used as an example, chicken was a luxury item in the 1960s, often being eaten only once or twice per year. The nature of chicken production has changed and is now undertaken on an industrial scale, using bird types with much faster growth rates than traditional breeds. As a consequence, the price of chicken meat has reduced dramatically putting it within reach of most consumers. However, the changes in production have had consequences for bird health and welfare and for public health, as will be discussed later. Chicken is now viewed as an everyday food, which is still increasing in popularity. Current estimates show that ~80% of chickens on sale in the wider EU are *Campylobacter*-positive at point of sale (EFSA).

The public health risk from chicken: Chicken contaminated with *Campylobacter* poses two health risks. Surface contamination levels are high and can reach 10^9 cells/carcass although most carry a few million cells[8,9]. Such high levels of *Campylobacter* lead to cross-contamination in catering and this is an important risk factor identified in outbreaks[10]. The high bacterial load is very different from that found in red meat and is probably a consequence of the speed of slaughtering and the fact that chicken carcasses and portions are generally wrapped, keeping meat surfaces moist, facilitating *Campylobacter* survival.

There is another risk associated with chicken. Unlike the tissues of other food animals, which are usually free from bacteria, and which are often eaten with only minimal cooking, *Campylobacter* cells can be isolated from deep chicken muscle and liver tissues. Frequency of isolation from muscle ranges from 5-30%[11]. Not all data published to date are unequivocal but there is now enough evidence to suggest that this is a genuine phenomenon. Isolation rates from liver are higher and can exceed 70% of samples tested[12]. The higher levels are associated with signs of liver disease[13] and *C. jejuni* can cause a condition known as vibrionic hepatitis, particularly in birds suffering from an endemic viral disease. Research groups in New Zealand and Switzerland are reporting high levels (>1000 cells/g tissue) of liver contamination[14]. There have been many chicken liver-associated outbreaks of *Campylobacter* infection in the UK in the past few years. A US research group has reported finding that 12% of blood samples taken from broiler chickens at slaughter were *Campylobacter*-positive[15]. Liverpool scientists hypothesise that muscle contamination may be the result of bacteraemia, with the bacteria being trapped in small blood vessels when the birds are bled.

The internal contamination of edible tissues poses a major public health threat, as the bacteria may be better able to survive cooking. Even if only a few *Campylobacter* cells inside part-cooked

liver are able to escape the lethal effects of gastric acidity and reach the intestine they will be sufficient to establish infection.

Sources of *Campylobacter* in chickens and risk factors for infection: The public health importance of *Campylobacter* infection of chickens means that there have been many studies on how the animals acquire the bacteria. Risk factors vary from paper-to-paper and between countries. However, there is general agreement that the most important source of infection is the farm environment[16]. It is common to isolate *Campylobacter* from the land around broiler houses and especially from damp areas and puddles. Most warm-blooded wild animals are also *Campylobacter*-positive.

Wild animals and insects as sources of *Campylobacter*: Wild animals act largely as an indirect source of flock infection through environmental contamination. Farms with mixed animal species have an increased risk of broiler flock infection with *Campylobacter* because farm staff may transmit the bacteria from other food animals to chickens. This undermines biosecurity, and a potentially important control measure is to rear chickens on mono-specific farms. Anti-*Salmonella* control measures which prevent the access of wild birds and rodents will contribute to protecting flocks from *Campylobacter* colonisation too. Work from Scandinavia has shown that in summer the ingress of contaminated flies into broiler houses is a risk factor for infection of the birds with *Campylobacter*. The use of fly screens over air inlets has been found to lower infection rates[17]. To date, work has not been done elsewhere but this is a potentially important control measure.

Infected flocks can have many *Campylobacter* sub-types: A number of different *Campylobacter* sub-types can be isolated from a broiler flock, and even from the same bird[18]. In general, however, one or two sub-types dominate. It is not known whether the different sub-types indicate entry of two different bacteria into the flock or changes in the original strain. The main event in the infection of a broiler flock is the establishment of the bacterium in the first bird(s). Passage through a chicken greatly increases the ability of *Campylobacter* to infect subsequent birds[19]. Spread can be very rapid in a newly infected flock, and most birds will be *Campylobacter*-positive within a few days of the initial colonisation event[20]. A major component of any control strategy must therefore be to prevent *Campylobacter* from the environment entering the broiler house. Given the current high rates of *Campylobacter*-positive chickens worldwide, with the exception of Scandinavia, maintenance of good biosecurity is something that the poultry industry finds challenging and other interventions will be needed to protect housed birds.

Thinning: An important risk factor for housed birds is 'thinning'[21]. Many poultry companies in the EU carry out this practice, largely for economic reasons. Broiler houses are stocked with numbers of birds which would be above the recommendation for stocking density if they all remained until slaughter. To overcome this, at approximately 5 weeks of age, a cohort (~30%) of birds is removed for slaughter, with the remainder being kept for around one week further. Thinning provides producers with the necessary flexibility to react quickly to the demands of the fresh retail market and may lower the carbon footprint of chicken production. However, thinning has important public health implications, in relation to contamination introduced by catchers and on crates. The potential ingress of *Campylobacter* is compounded by the fact that the birds remaining in houses will be stressed as a result of the thinning process. This may render them more susceptible to *Campylobacter*. The role of bird stress in the *Campylobacter* infection process is discussed below.

The effects of feed withdrawal: An important hygiene problem in broiler processing is the accidental contamination of the carcass at slaughter by gut contents, particularly faecal material leading to the spread of *Campylobacter*. To reduce the danger, feed is withdrawn some time before catching, whether at thinning or at final depopulation. Fasting periods of 4-10 hours have been recommended. However, the overall period without feed will be longer than this because of the time taken to load and transport the birds to the processing plant and time spent in the lairage before slaughter. There is continuing debate about whether these fasting times are beneficial.

Reducing the gut contents will lower the pressure on the intestines and help prevent leakage of contents on to the carcass if the gut is broken during evisceration. However, even prolonged feed withdrawal will not completely prevent defaecation occurring during ante mortem handling. Removing feed, or both feed and water, have similar effects on gut contents. Most reduction in weight occurs in the crop and least in the caeca and cloaca. An important finding is that the contents of most parts of the gut, but particularly those of the crop and cloaca, get wetter with longer deprivation. In contrast, caecal contents become slightly drier. Fasting tends to progressively increase the number of *Campylobacter* in the gut but especially in the caeca and cloaca[22].

Feed withdrawal will not eliminate cross-contamination of the plumage of live birds with faecal matter during transport. Moreover, it may also have unforeseen adverse effects by inducing stress, which may pre-dispose birds to *Campylobacter* infection. The modern broiler chicken has been bred to eat. Work with *Salmonella* found that birds can be systemically infected very rapidly (within 2 hours) after exposure to sources of infection. Given the commonness of *Campylobacter* in poultry it is probable that infection with this bacterium will be equally rapid. Feed withdrawal may also affect the microbes in the gut and has a particular effect on lactobacilli. These bacteria have the ability to prevent/reduce intestinal colonisation with *Campylobacter*. A study, which examined the effects of stress in young monkeys, found that this was associated with a reduction in levels of lactic acid bacteria in the gut[23]. Many of the stressed animals became infected with *C. jejuni*, which was endemic in the colony. It is also of interest that longer feed withdrawal times (up to 24 hours) are associated with a higher prevalence of chickens testing positive for *C. jejuni* in crop samples before slaughter [24]. A key question is does the possible increased risks of gut breakage and greater susceptibility to infection outweigh perceived benefits on lower carcass contamination levels with *Campylobacter*? It is reasonable to theorise that birds remaining after thinning might be more susceptible to infection as a result of a combination of stress induced by disturbance and feed withdrawal.

Broiler production systems, bird health and welfare: Until recently, work on the identification of risk factors for infection of broiler chickens with *Campylobacter* has focused on sources of infection and associated risks. This is, of course, valuable but has largely missed two important components of the chicken-*Campylobacter* dynamic, the bird and the conditions in which it is grown. Commercial animals are mainly reared in housed (broiler) systems and these are discussed below. There is also a large market for chickens reared extensively in organic or free-range systems. In these, chickens have access to the natural environment. Given that the environment is the principal source of *Campylobacter* for chickens, it is not surprising that most animals grown outside are positive for these bacteria by the time they reach slaughter age.

There is a need for the risk from extensive systems to be properly audited to take account of all contamination scenarios. It needs to be established whether extensively reared birds pose the same public health risk as ones grown inside. If risk is based solely on contamination of carcass

surfaces then such birds are high risk. However, if other factors such as contamination of edible tissues are taken into account, the risk from extensively reared birds may be lower. This is based on the premise that birds reared outside have better welfare than ones in intensive production. If this is the case, then research suggests that in such birds *Campylobacter* is more likely to remain in gut and only contaminate carcass surfaces at slaughter.

Chickens that are housed are grown essentially in two commercial systems. Standard broiler production uses rapidly growing birds, stocked at high density, which reach slaughter weight (2.2 Kg live weight) at 35-37 days. The second system is one where higher welfare standards are applied. Birds are stocked at lower density, have environmental enrichment, including windows, and generally grow more slowly, reaching slaughter weight up to 20 days later than birds in standard systems. Slower growing birds of the type used in the higher welfare systems are generally healthier, having lower levels of endemic disease and contact dermatitis[25]. This is important because work by Liverpool and Newcastle scientists found that endemic *E. coli* infections and high levels of hock marks and pododermatitis (manifestations of contact dermatitis and indications of poor gut health) are important risk factors for *Campylobacter* in commercial flocks[26]. Given these data, it would not be unreasonable to assume that 'slower-growing' broiler phenotypes are less likely to be *Campylobacter*-positive[27]. Work is in progress at Liverpool to determine the *Campylobacter* status of broilers grown under different systems, including those with higher welfare.

At present, it is not an economically viable proposition for the international poultry industry to switch completely to higher welfare systems and/or use 'slower-growing' birds. However, work is in progress at Liverpool to determine what are the key gut health and immunological differences between 'slow' and 'fast' broilers. This might make it possible to improve the 'fast' birds making them not only more resistant to *Campylobacter* but also to important endemic pathogens like *E. coli*. Some poultry companies are now growing the 'fast' birds in higher welfare systems, to make this production type more economically viable. If house environment and environmental enrichment are important factors in the *Campylobacter* infection process, levels of this pathogen in these birds will be lower. If not, then the bird and not its environment plays the major role in determining *Campylobacter* status. There is an urgent animal welfare and public health need to determine the effects of selection for rapid growth on the gut environment and disease resistance.

Chicken stress responses and *Campylobacter*: Host stress plays a very important role in regulating the *in vivo* behaviour of *Campylobacter*. Unpublished work by the author found that in birds that are subject to either simulated acute (catching or transport) or chronic (a poor production environment) stress, *C. jejuni* can be highly invasive and can adopt a pathogen-like behaviour, as has been shown *in vitro*[28]. In birds where chronic stress was modelled by giving them corticosterone in drinking water for two days, *C. jejuni* caused profound and potentially fatal diarrhoea and was recovered from liver tissues with high frequency. Chronic stress leads to immunosuppression, rendering the bird less able to resist *Campylobacter* and keep it in the gut[29].

Acute stress leads to the release of high levels of noradrenaline into the bird's intestine. This markedly changes the intestinal environment, rapidly lowering the levels of potentially protective bacteria like lactobacilli and changing the permeability of the gut wall[30]. Noradrenaline can also act as an iron-capture mechanism for *Campylobacter*. The hormone will take iron from gut contents and/or the host and transport it back to receptor cells on the bacterial cell surface. Iron is then taken into the cell. When *Campylobacter* is iron-rich its growth

rate is markedly increased[31] and it is common practice to find that levels of *Campylobacter* are higher in birds that have been caught and transported compared to ones from the same flock left on the farm[32]. Many virulence genes in *Campylobacter* are iron-regulated and preliminary work by the author showed that cells of *C. jejuni* grown in the presence of noradrenaline colonise chickens better and are also more invasive being isolated with high frequency of liver tissues.

The interactions between *Campylobacter* and its chicken host: The interaction of *Campylobacter* with its chicken host is a dynamic one where both partners are changing over time. The bacteria continue to involve but perhaps more importantly, chickens reared for food are being selected to grow and put on weight ever more quickly. There is a research and public health need to determine how changes in broiler phenotypes have affected the interaction of the bird with *Campylobacter*. Laboratory studies indicate that even relatively small changes in the welfare and/or immune status of birds brings about marked alterations in the *in vivo* behaviour of *Campylobacter*.

Many studies to inform the infection biology of *Campylobacter* in chickens have been undertaken in ideal conditions in the laboratory, often using types of chickens very different to those grown commercially. In these circumstances, the bacteria largely behave as harmless 'commensals'. Importantly, the conditions in the laboratory bear little or no resemblance to those found in commercial production. Like many other 'commensals', *Campylobacter* can show pathogen-like behaviours if the host is compromised.

The balance between the broiler chicken and *Campylobacter* is a fine one and it is essential for improvement of public health that work is undertaken to fully understand how modern chickens interact with *Campylobacter* and the public health consequences of this.

Control of *Campylobacter* in chickens: The international poultry industry faces a major challenge in trying to control *Campylobacter* in both extensive and intensive production systems. This matter is particularly pressing in the EU as it is likely that sometime in the near future a baseline figure will be set for each member state, as has been done for *Salmonella*. Past work has shown that *Campylobacter* control is possible for housed birds by strict observance of biosecurity by farm staff[33]. This included changing footwear and clothing each time the broiler flock is visited, which can be both laborious and time-consuming. The current high levels of chickens that are positive for *Campylobacter* at retail clearly indicate that either biosecurity is not being properly applied and/or that measures that were once successful no longer work as well, possibly because the modern broiler is more susceptible to infection. Biosecurity needs support. Many options have been explored and on-farm these include vaccination, feed treatments and/or probiotic bacteria and treatment of birds with viruses (phages) that kill *Campylobacter*. All have had some measure of success but, as yet, none have been shown to be sufficiently effective to significantly improve public health. It may well that a combination of measures may be necessary.

Work is in progress to determine whether it will be possible to breed chickens that are resistant to *Campylobacter*. Some inbred chicken lines have shown differences in *Campylobacter* carriage rates but these animals grow very slowly and are currently not commercially viable. However, this could be an attractive option as it could help to better protect extensively reared birds where biosecurity is not an option.

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