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Campylobacter as the main zoonotic pathogen in poultry and strategies for its control

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Summary

Campylobacter spp. are the most frequent cause for infectious enteritis in developed countries. Poultry may harbour this bacterium symptomlessly in the intestines, and live animal transport and slaughter procedures may ultimately result in contamination of carcasses with this pathogen. There is evidence that 20–30% of human Campylobacteriosis cases in the EU are linked to poultry meat, although conditions prevalent in the food chain, such as low temperature and presence of oxygen, are not favourable for multiplication or survival of this bacterium. This paper characterizes the biological hazard ‚*Campylobacter*' and reviews current and proposed control strategies, with a focus on on-farm measures.

Zusammenfassung

Campylobacter - die häufigsten Zoonoseerreger beim Geflügel und deren Bekämpfung

Campylobacter spp. sind die häufigsten Erreger von infektiöser Enteritis in Industrieländern. Das Bakterium kann von Geflügel syptomlos im Darm beherbergt werden, und der Lebendtiertransport und der Schlachtvorgang können letzlich zu einer Kontamination der Schlachtkörper mit diesem Pathogenen führen. Es wird angenommen, dass 20–30 % der humanen *Campylobacter*iosefälle in der EU auf Geflügelfleisch zurückgeführt werden können, obwohl die Umweltbedingungen bei der Fleischgewinnung (niedrige Temperatur, Sauerstoff-Atmosphäre) für die Vermehrung oder das Überleben dieses Bakteriums ungünstig sind. In der vorliegenden Arbeit wird neben einer Charakterisierung der biologischen Gefahr „*Campylobacter*" auch eine Übersicht zu gegenwärtigen und geplanten Kontrollmassnahmen gegeben, wobei der Schwerpunkt auf Massnahmen im Herkunftsbetrieb liegt.

Introduction

Campylobacter spp. are bacteria that commonly infect a broad range of livestock species, pets and wild animals. In poultry they tend to multiply in large numbers in the hindgut, principally the caeca. Of the various *Campylobacter* species, *C. jejuni* is the most common in poultry and is currently not considered to be pathogenic in poultry, although as the science advances this view is changing. There are indications that plantar pododermatitis, carcass quality and litter quality are better on farms which tend to have *Campylobacter*-negative stock. Although the reason for this is unclear, it may be that management favouring dry litter reduces the risk of infection and/or transmission in the flock (McCULLIN, 2004). Whereas there are generally neither clinical symptoms norpathological anatomical *post mortem* lesions in *Campylobacter*-positive birds, the organism can be isolated from caecal contents, cloacal swabs or composite faeces, and from liver under certain conditions (see below).

Current knowledge and knowledge gaps on *Campylobacter* in poultry

Campylobacter as a poultry-associated zoonotic pathogen

Campylobacter is the most common bacterial cause of diarrhoeal disease in the developed world. It is estimated to infect 1% of the EU population each year and in the UK in 2010 it is believed that ~700,000 people were infected with ~200 deaths (TAM et al.,

2012). Extreme outcomes of infection include irritable bowel syndrome, arthritis and paralysis. An EFSA publication (EFSA, 2010a) stated that the contamination figure for *Campylobacter*-colonised broiler batches was 71.2% and that of *Campylobacter*-contaminated broiler carcasses was 75.8%. A recent EFSA Scientific Opinion on *Campylobacter* in broiler meat production (EFSA, 2010b) stated that: ‚It is estimated that there are approximately nine million cases of human Campylobacteriosis per year in the EU27. The disease burden of Campylobacteriosis and its sequelae is 0.35 million disability adjusted life years (DALYs) per year and total annual costs are 2.4 billion €. Broiler meat may account for 20% to 30% of these, while 50% to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens). The public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat.' The authorship of the present paper strongly believes that reducing chicken colonisation by *Campylobacter* on-farm is essential for controlling human infection caused by this pathogen.

Chicken carcass contamination presents two threats. Surface levels as high as 10^9 cfu/bird pose a cross-contamination risk (JØRGENSEN et al., 2002) and extra-intestinal spread to sites such as muscle and liver presents a greater risk, as the chance that *Campylobacter* may survive cooking is greater (BERNDTSON et al., 1992; LUBER and BARTELT, 2007; SCHERER and BARTELT, 2006). Past work has shown that extra-intestinal spread of *Campylobacter* in chickens *in vivo* is related to bird genotype/growth rate and production systems and by host responses to acute and chronic stress. Co-infection with endemic disease agents such as avian pathogen *Escherichia coli* (APEC) will also increase the likelihood of infection of edible tissues.

Campylobacter in broilers from environment to colonisation

Campylobacter is found in the natural environment and in a wide variety of animals. It is generally believed that indoor-reared birds acquire *Campylobacter* from the external environment around the broiler house by horizontal transmission (NESBIT et al., 2001) although this is the subject of some debate (COLLES et al., 2008). The processes leading to chicken colonisation by *Campylobacter* are multi-factorial and involve the frequency and type of exposure to different fomites and vectors in their environment, the potential of the bacteria to establish in the gut and the susceptibility of the host, which may be affected by the environment in which the bird is reared. *Campylobacter* is most abundant in the caecum. Here the organism forms a major component of the microbiota.

There is reason to believe that housed broilers are repeatedly challenged with low numbers of *Campylobacter* from the external environment during rearing and that colonisation occurs when the bacterial dose-gastrointestinal environment relationship is permissive. Flock events in management influence bird susceptibility by affecting the gut environment and hence the likelihood of colonisation (BULL et al., 2008; RUSHTON et al., 2008). There have been many investigations of *Campylobacter* colonisation of broilers and a range of risks found, such as weather, presence of other livestock and production system. A longitudinal study in the UK (BULL et al., 2008) and subsequent analysis (RUSHTON et al., 2008) used modelling to investigate how *Campylobacter* colonisation is related to bird management. Work in Northern Europe shows that, even in countries like the UK where production is highly intensive, it is possible for farmers to rear *Campylobacter*-negative housed broilers, often repeatedly, especially if they are sampled pre-thinning and in Spring/Winter (BULL et al., 2008; RUSHTON et al., 2008).

The summer peak in *Campylobacter* levels in broilers

Previous work in Northern Europe has shown that housed flocks are significantly more likely to be *Campylobacter*-positive in summer (BULL et al., 2008; PATRICK et al., 2004; GUERIN et al., 2008), when ambient temperatures were high around the time of slaughter and also when it was raining at this time (RUSHTON et al., 2008). The former is likely to be related to increased airborne transmission of *Campylobacter* due to higher airflows into the house. The bacteria may be free in air or, more importantly, associated with flies (HALD et al., 2004).

Heat stress in the birds may also be important in summer. Rainfall-associated risks may be related to better *Campylobacter* survival in air because of raised relative humidity (RH) (LINE et al., 1997). In the North EU, a major challenge to industry is to protect birds in summer. In the framework of an EU project (Cam Con) the existence of a summer peak is currently being investigated, focusing on the UK and Spain, whilst in another international project (CamChain) - that has started in July of 2012 - a study of seasonality in South East Asia is being undertaken. It is conceivable that colonisation potential is governed by prior environmental exposure of *Campylobacter*. Indeed, past work (HUMPHREY, 1986) found that cells of *C. jejuni* exposed to cold grew less well at 42 °C, chicken body temperature.

The ‚*Campylobacter*-free' period

Campylobacter colonisation of housed commercial birds is not generally detectable until ~3–4 weeks after house filling, although extensively reared birds can be positive earlier (ALLEN et al., 2011). The respective and combined roles of maternal antibodies and potentially competitive chicken gut microbiota in

protecting young chicks in the first few weeks of life is currently being investigated (CAWTHRAW and NEWELL, 2010).

A major data gap exists on the timing of entry of *Campylobacter* into a broiler house and initial colonisation of the flock. Similarly, data on rate of spread of *Campylobacter* are lacking and given proposed changes to bird stocking density in the EU, it is important to establish how these affect transmission of these bacteria. Once colonised, within-flock spread is influenced by water management and drinker type is a risk factor for colonisation (RUSHTON et al., 2008). LINE (2006) found that the RH of the in-house air also influenced colonisation and spread with both being higher when it was high. A study of the epidemiology of *Campylobacter* in broilers to be fully comprehensive, should gather data on the in-house environment such as litter pH and moisture content and RH of the house air.

Bird health and welfare and *Campylobacter* colonisation

So far, little attention has been focused on whether bird health and/or welfare affect the host-*Campylobacter* dynamic. Thus potentially important components of the infection dynamic have been ignored. Clearly, whether or not Campylobacter cells entering the broiler can successfully colonise the intestine of birds will be affected by the state of the host. In a recent UK study, *Campylobacter*-negative flocks were compared with full or partially colonised ones. This revealed that those with poor gut health as indicated by higher than usual levels of hock marks and/or pododermatitis or where birds were infected with APEC were significantly more likely to be *Campylobacter*-positive, particularly at a high level (BULL et al., 2008; RUSHTON et al., 2008). In addition, work in Norway (SKÅNSENG et al., 2007) has shown an association between necrotic enteritis and *Campylobacter* in broilers. It is not yet known if the links were causal or if they indicate a common environmental factor or they are a marker of general poor bird management.

Partial depopulation as a risk for *Campylobacter* infection

Many EU poultry producers practice partial depopulation or ‚thinning' at 5–7 days before most birds will be slaughtered. Around 30% of the birds are removed at this stage so that farmers can conform to ‚end-of-life' stocking density guidelines with the remaining birds. It is generally believed that thinning increases the *Campylobacter* colonisation rates of the birds left behind but data currently available are equivocal. In this context it is important to realize that this imbalance may affect the risks of ‚thinning', which has economic advantages for the farmer and also lowers the carbon footprint of intensive chicken production, during the longitudinal studies.

Extra-intestinal spread of *Campylobacter* in chickens

The ability of some *Campylobacter* strains to leave the gut and infect edible tissues is increasingly being recognised as a public health threat. In essence, *Campylobacter* in chickens poses two threats. Contamination levels are high, and cross-contamination during catering is common and is an infection risk (COGAN et al., 1999; JØRGENSEN et al., 2002). Of greater importance, is that *Campylobacter* is found in deep muscle of up to 27% of chickens (SCHERER et al., 2006; LUBER and BARTELT, 2007) increasing chances of survival during cooking. This may explain why undercooked chicken is an important vehicle for infection. *Campylobacter* spp. have also been isolated from liver tissues from both naturally and artificially infected birds (KNUDSEN et al., 2006; JENNINGS et al., 2011). Research suggests that host stress and innate immune responses can work either singly or together in the bird to create invasive *Campylobacter* phenotypes (COGAN et al., 2007; REES et al., 2008). Thus in the South EU, heat stress in the birds, which may lead to raised noradrenaline and corticosterone levels, could be an important factor in the colonisation process (HUMPHREY, 2006).

The UK, in particular, has seen a marked rise in *Campylobacter* outbreaks caused by chicken liver or products derived from it. *Campylobacter* contamination levels in liver can exceed 10^4/g and the bacteria survive short exposures to temperatures as high as 70 °C (WHYTE et al., 2006). UK research initiatives seek to determine where liver contamination/infection occurs. Data showing that diseased livers have higher *Campylobacter* levels than healthy ones suggests that bird health may be an important component of this process. Unfortunately, to date, nothing is known about the mechanisms used by *Campylobacter* to leave the chicken gut and infect edible tissues.

On-farm intervention measures

The EFSA scientific opinion referred to earlier states that ‚Biosecurity measures are considered essential to prevent flock colonization with *Campylobacter*.' Currently - through CamCon and UK-funded projects - many physical on-farm interventions are being examined. Maintenance of biosecurity at the level required to exclude *Campylobacter* is difficult for the poultry industry and requires support. Measures to remove *Campylobacter* such as phage treatment may also play a role, as might in ovo vaccination.

A range of pre- and probiotics have been tried as anti-*Campylobacter* treatments, with varying levels of success. It is currently being investigated to what extent potentially probiotic bacteria and potential prebiotic diets are effective mitigation strategies, not only against *Campylobacter* (in the laboratory and on-farm), but also to positively influence bird gut

health and the gut microbiome. Both play a major role in susceptibility of birds to *Campylobacter* and also influence the *in vivo* behaviour of these bacteria.

Survival of *Campylobacter* in the food chain

It is not well understood how *Campylobacter* survives exposure to hostile conditions, nor whether exposure to them affects virulence; still less is known about the molecular mechanisms which underpin its ability to survive. Most *Campylobacter* infections are food-borne and important vehicles include cooked chicken, barbecued meat and raw or improperly pasteurised milk (HUMPHREY et al., 2007). Chicken-associated *Campylobacter* cells are exposed to high temperature during scalding and must survive periods at low temperature. It is highly relevant to study survival in both natural and processing environments and to pay particular attention to exposure to high or low temperatures.

In the farm environment, *Campylobacter* are found in livestock, wildlife and the environment (soils and water). There is limited, piecemeal, understanding of *Campylobacter* ecology in natural and production environments. *Campylobacter* has been considered to be fragile outside the host gut, yet in practice it can survive well in moist farm environments and on chicken carcasses, possibly through interaction with other members of microbial community present. *In vitro* studies show that *C. jejuni* co-cultured with strains of *Pseudomonas* spp., amoebae and algae or in biofilms, can survive in normally adverse aerobic/acidic conditions (MURPHY et al., 2006). The role of microbial communities on *Campylobacter* ecology in natural and farm environments has not been studied.

Exposure of *C. jejuni* to stresses in the food chain

Potential food chain intervention strategies will be sub-standard unless there is more knowledge about the fundamental biology of *Campylobacter*, especially in environments relevant to food production. There is a lack of authoritative data, particularly at the molecular level, on the responses of *Campylobacter* to food chain stressors. Earlier studies have shown that *Campylobacter* isolates differ in survival rates in hostile environments (HUMPHREY, 1986). Other work showed that *C. jejuni* isolates differ in virulence in animals and *in vitro* assays (CALDERÓN-GÓMEZ et al., 2009). What has not been done is to establish whether there is a relationship between environmental resilience, stress responses at the molecular level and virulence, as has been shown to be the case for *Salmonella* Enteritidis (JØRGENSEN et al., 2000). However, it is essential that when food chain interventions, which do not eradicate zoonotic pathogens, are planned the consequences of such actions are fully understood, particularly if there is a possibility that pathogen virulence and subsequent survival in the food chain might be enhanced.

Refrigeration is central to the preparation, storage and distribution of chicken and chicken products. *Campylobacter* lack RpoS homologues and cold shock proteins which might be expected to limit its responses to low temperature. However, it survives treatments in the chicken production chain, including exposure to water at 5–15 °C during slaughter and prolonged storage (up to ten days) at low temperature (~1–6 °C). The principal zoonotic *Campylobacter* spp., *C. jejuni* and *C. coli*, have a minimum growth temperature of ~30 °C and were thought to be almost inactive in the cold. This view is influenced by the parameters chosen to assess responses to cold. Recent work measured electron transport activity in 19 °*C. jejuni* strains and showed an 87% decline in activity after 10 min at 6 °C and a 99% fall by 24 h (HUGHES et al., 2010). However, *C. jejuni* retains metabolic activity, including de novo protein synthesis, chemotaxis and aerotaxis, at 4 °C. Transient increases in colony counts were seen in *C. jejuni* populations held at 4 °C, indicating that it may grow at low temperatures or switch between culturable or non-culturable states. Cold enrichment at 4 °C improved the isolation rate of *C. jejuni* from faeces. Most (96%) *C. jejuni* cells held at 4 °C remained spiral, indicating probable viability. Although *C. jejuni* is physiologically active at 4 °C, it has no obvious homologue of the major cold shock protein of *E. coli*, CspA.

There has not been a comprehensive study of *Campylobacter* behaviour at low temperature, few published data exist on responses to cold at the molecular level and few strains have been studied. One study (STINTZI, 2003) examined a 10 min exposure of *C. jejuni* 11168 to 4 °C and found that expression of many genes was significantly changed compared to cells held at 37 °C. Other work with 11168 examined exposures of up to seven days. Genes involved in energy metabolism were more active at 5 °C than at 25 °C.

Equally relevant would be to examine survival at high ambient temperatures and under regimes, which simulate under-cooking. Most foods in *Campylobacter* infection are heated prior to consumption and there has been almost no work at potentially lethal temperatures and the mechanisms that permit *C. jejuni* to survive such exposures are poorly understood. The latter is currently being investigated in the framework of the CamChain project (see above). One paper (KONKEL et al., 1998) showed that immediate exposure to 55 °C did not permit the expression of heat shock and starvation (dnaK, htpG, groEL) and oxidative stress response (ahpC, sodB) genes.

Differences in survival profiles at high and low temperature in different *Campylobacter* isolates

Populations of *Campylobacter* are highly variable. For instance, various studies (CHAN et al., 2001) have shown that human isolates survived better at 4 °C

than poultry-derived strains. In contrast, the relative degree of tolerance to freezing at -20 °C, and to freeze-thawing, was strain-specific but independent of whether the strain was sourced from chickens or humans. The mechanisms underlying the different survival profiles are unknown. Also, differences in heat tolerance between *C. jejuni* isolates have been observed. It would seem to be essential to determine whether outbreaks in humans are influenced by significant under-heating or if the *Campylobacter* isolates involved have an enhanced ability to survive such exposures and to cause infection.

Does the food chain select more virulent strains of *Campylobacter*?

No chicken carcass treatments examined to date removed all the *Campylobacter* cells present. There is a danger that treatments may leave behind a more robust and potentially more virulent population of these bacteria. In the CamChain project (see above) population structures are being examined and the virulence of the major population members before and after processing determined, once the efficacy of on-farm interventions has been established. It has also been shown that *C. jejuni* isolates exposed to freezing can quickly recover virulence. Carcasses carry many different *Campylobacter* sub-types and populations change during processing (LIENAU et al., 2007). It is unknown if this is also a reflection of differences in inherent resistance to changes in temperature or responses to early stages in the food chain facilitating subsequent survival.

It was found that dominant *Campylobacter* types on live birds formed only a minor component of the eventual carcass population (NEWELL et al., 2001). It is unclear if this results from different heat- and/or cold-resistance. It should be recognised that *Campylobacter* is very different from *Salmonella*. However, does better environmental survival of *Campylobacter* also mean enhanced virulence, which has been shown to be true for *Salmonella*? (JØRGENSEN et al., 2000). Work on *L. monocytogenes* found that cells exposed to 4 °C became more virulent. Flagella are important in the virulence of *C. jejuni* and the fact that there are indications in the scientific literature (STINTZI, 2003) that the transcription of genes for flagellar proteins (Cj0697, Cj0042 and Cj1462) was reproducibly increased at 6 °C might suggest enhanced virulence.

Given the above data it would be reasonable to assume that *C. jejuni* strains that are less stress-resistant would show reduced virulence. There has been surprisingly little work in this area and only one study with cold-exposed *Campylobacter*. *C. jejuni* was able to invade CaCo-2 cells after 12 days at 6 °C; whereas exposure to higher temperatures (12 °C and 25 °C), led to a rapid drop in this ability (KONKEL et al., 1998). Cells exposed to 55 °C for 3 min showed significantly reduced invasion into CaCo-2 cells.

The role of flies in the transmission of *Campylobacter*

Recent studies carried out in Denmark (HALD et al., 2004, 2007, 2008) have proven that flies – in particular the house fly, exhibit a significant role in transmitting *Campylobacter* from farm environment to broiler houses during summer when the insect population is abundant. When ingress of flies to broiler houses was prevented by fine meshed screens, the prevalence of *Campylobacter* positive flocks at slaughter was reduced.

Control of *Campylobacter* along the food chain – summarised

Control of *Campylobacter* should primarily aim at reducing the exposure of consumers to this pathogen. In theory, a multitude of measures are possible, yet not all can be implemented in practice or are sufficiently effective.

Pre-harvest

The control of *Campylobacter* at pre-harvest level includes: (1) reducing the exposure of chickens to *Campylobacter*, which is in essence the task of biosecurity and (2) prevention of colonization of chickens´ intestines with the bacterium. These measures should result in not only a low flock prevalence, but also a lower within-flock prevalence and finally, in lower *Campylobacter* concentrations in faeces. Despite the efforts in the last decades, there is no single universally applicable or sufficiently effective measure available.

Fresh meat chain: slaughter, chilling, cutting and packaging

The slaughter process of poultry differs from that of ruminants in the way that it is a wet-slaughter procedure, highly-mechanized, with high throughput. Both scalding and evisceration play a significant role in faecal contamination of the carcass, and the feather follicles and crevices of skin and subcutaneous tissues create niches where contaminant bacteria can be entrapped and are protected from environmental adverse factors. Dry (e.g. air-blast) chilling is known to reduce the number of culturable *Campylobacter*, but given the protective effect of tissue structures and the pressure exerted by selective microbiological cultural media on presumably impaired bacterial cells, it is more likely that a certain fraction of bacteria will remain viable, although they cannot be detected by cultural microbiology.

In general, the high minimum growth temperature and the sensitivity to oxygen should neither allow multiplication nor prolonged survival of the bacterium in the fresh meat chain. There is evidence that when poultry carcasses are traded only in deep-frozen

condition, this is associated with a decrease in human Campylobacteriosis, although there may be some confounders in these studies. However, the micro-ecology of meat, in particular the presence of oxygen consuming *Pseudomonas*, may allow survival and recovery of *Campylobacter* post-slaughter (HILBERT et al., 2010).

Slaughter of *Campylobacter* negative flocks before positive ones (logistic slaughter) will have only limited effect on the overall carcass prevalence when the flock prevalence is high, and the time needed from sampling of flocks to obtaining results and arranging the order of slaughter introduces some uncertainty.

Food handling and preparation

Food handling and preparation can be described as a sequence of surface-surface contact events, and food technology measures, such as temperature, pH adjustment, organic acids and preservatives. The transfer of *Campylobacter* has been the subject of several studies, both of experimental nature or following a modelling approach. In essence, each surface-surface contact, i.e. contamination event, will transfer only a fraction of bacteria. Irrespective if the contaminated foodstuff is ready-to-eat or receives a treatment lethal for *Campylobacter*, its concentration in a food serving will be lower than in the raw foods before processing. Knowledge about the retail-to-consumption chain and moreover, a quantitative description how *Campylobacter* numbers are affected during this chain, can allow to establish performance objectives, based on a predefined food safety objective (FSO).

Concluding remarks

Campylobacter jejuni and *C. coli* remain major zoonotic pathogens worldwide and infection is mainly associated with the consumption of under-cooked poultry and meat products and raw milk. *Campylobacter* presents a major challenge to the food industry and regulatory authorities. One important contributory factor is the fact that in most food animals, *Campylobacter* acts as a harmless gut commensal and this symptomless carriage is often life-long. There is increasing evidence, however, that in broiler chickens, in particular, some *Campylobacter* strains can cause disease and the processes that lead to this state need to be understood. *Campylobacter* was only properly recognised as a human pathogen in the late 1970s and we have yet to fully understand its epidemiology in man and food animals and its survival capabilities in the environment. The latter is especially important as a contaminated environment is a major direct and indirect source of human infections.

Disclosure

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