**Campylobacter and Poultry**

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**Introduction**

This paper attempts to provide an insight into managing *Campylobacter* within the chicken meat industry. The paper consists of three sections – a section that provides the basic information on the biology of this organism, a section that presents the case for managing the levels of *Campylobacter* as opposed to seeking freedom from *Campylobacter* and finally a selective review of the published literature on methods to control *Campylobacter* spp. on the farm.

**Disease and basic biology**

Infections of humans caused by campylobacters are a major cause of food borne illnesses in many countries, including Australia (1) and most other developed countries (2). *Campylobacter jejuni* and *Campylobacter coli* cause the majority of human disease (3). Unlike other agents of bacterial food-borne disease such as *Salmonella enterica*, *Campylobacter* sp. infections are largely sporadic rather than occurring in outbreaks (4), making it difficult to understand the transmission events that result in human disease (3). However, it has to be recognised that poultry meat has been consistently identified as a major, but not sole source, of the organism entering the human population (5).

There is a considerable cost to the community of campylobacteriosis. In Australia, it has been estimated that *Campylobacter* causes 223,000 cases of gastro-enteritis a year, with over 50,000 of those cases being linked to chicken meat (6). In 1995, it was estimated that each case of food-borne campylobacteriosis in the USA was costing (on average) somewhere between US$350-580 (7). On this basis, it is reasonable to assume that each Australian case of campylobacteriosis in 2010 would have resulted in a cost of around $500 (includes medical costs, medication and lost productivity). Hence, this single food-borne pathogen could be costing Australian society around $250 million annually.

There are some key biological facts that need to be understood. *Campylobacter jejuni/coli* can be highly infectious for humans with an ID90 of around 500 cells (8). On the broiler farm, *Campylobacter* spreads rapidly – all chickens in the shed can be positive within a few days of the first positive birds being detected (9). Importantly, chickens rarely shed the organism before 14 to 21 days of age (10).

*Campylobacter* are ubiquitous in the intestines of almost all poultry, farm animals, pets, wild animals and wild birds (11). It has to be emphasised that, with some exceptions, humans and monkeys are the only hosts that suffer adverse effects following colonisation by *C. jejuni/coli*. *Campylobacter* spp cannot multiply outside the host and survival in the environment is relatively poor (11). This relatively poor survival has to be balanced by recognising that large numbers of *Campylobacter* spp. are shed into the environment with the faeces of colonised animals (11).

There has been considerable debate about whether *Campylobacter* can enter a “viable but non-culturable” state, a resting or dormant stage induced by cell stress or starvation. This state is supposed to occur when the bacteria are exposed to the environment outside the animal host. The significance of this state in terms of disease transmission has long been questioned. Some studies have reported that “viable but non-culturable” *Campylobacter* can remain infective (12, 13) while other studies have reported that “viable but non-culturable” isolates are also non-infective (14, 15). While the issue has received little to no attention in the recent literature, it remains possible that “viable but non-culturable” forms of *Campylobacter* might exist and might be infective. Hence, there is a need to recognise this possibility when studying the environmental survival of *Campylobacter* in the environment.

**Management, not freedom, is the focus**

*C. jejuni* and *C. coli* are widely distributed in birds (16). It appears that chickens have a higher of *Campylobacter* than do wild birds (17). As it is possible that *Campylobacter* is simply part of the normal chicken gut flora of the chicken, the goal of freedom from *Campylobacter* is a difficult target. However, total freedom from *Campylobacter* is not necessary. European studies have shown that on-farm interventions can be very effective in delivering effective improvements in public health even if freedom from *Campylobacter* is not achieved. For example, a 2 log reduction in faecal *Campylobacter* counts on farm has been shown, by modelling, to reduce human infections by 75% (18). This same modelling approach showed that the combination of a 1 log reduction in faecal counts on farm and a 1 log reduction in the abattoir can achieve a 90% reduction in human infections (18). Hence, using Australian data, the development and validation on-farm of tools for reducing *Campylobacter* levels by 1 to 2 logs can realistically result in a lowering of human infections in...
Australia from 50,000 to 5,000 case per year and save around $225 million.

**Potential on-farm control strategies**

Any overall program that seeks to reduce the *Campylobacter* contamination of poultry products needs to have components across the full farm to consumer chain – ie controls that focus on-farm, within the processing plant and along the delivery chain that runs from the plant to the customer. This review has focussed on the on-farm component of the overall picture.

**Maternal Antibody:** Typically broilers become infected with *Campylobacter* at the same age as when maternal antibodies wane (19). Laboratory tests have shown that maternal antibodies can kill *C. jejuni* (19). All of this suggests that vaccinating broiler parents may reduce *Campylobacter* colonisation in the broilers. Against this logic is the fact that young chicks can be infected by less than 50 *C. jejuni* cells (20). Hence, Mead (10) has concluded that maternal antibodies are not effective at preventing colonisation in vivo.

**Biosecurity:** Controlled trials in the UK, that used increased biosecurity, have shown a reduced prevalence of *Campylobacter* infection in the birds close to pick up (from 80% to 40%) (21). The key intervention was a twice weekly replenishment of the disinfectant in the boot dips (21). The researchers concluded that the results were significant and effective (21). In contrast, an industry partner within the project had a far more pessimistic view, stating that the same results “indicate that it is difficult and probably impossible to guarantee keeping *Campylobacter* infection out of the poultry house” (22). The industry partner concluded that the key hygiene barrier, a step-over bench that forced a change of boots and overalls (supported by foot dips that were regularly replenished) might be an effective intervention strategy BUT the system was beyond the practical things that a farmer could realistically implement (22).

Other biosecurity studies have shown that key issues have been adoption of disinfection of boots (or clean boots), clean overalls and hand-washing when entering the shed (23), rapid removal of dead birds (24) and avoidance of “thinning” of flocks (23). Recently, a UK study has reported that while there is a low prevalence of *Campylobacter* positive flies around broiler sheds, the risk of transmission by flies may be high, particularly when fly populations are high in summer (25). Corry and Atabay (16) have concluded that the three key control measures are i) drinking water chlorination; ii) hygiene measures for visitors and workers and iii) control of wild birds, rodents and flies. In another UK study, “enhanced” and normal biosecurity were compared (26). The “enhanced” biosecurity measures were as follows:-

1. Vehicle cleaning and disinfection before farm entry
2. Catching crew provided with mobile mess/changing room
3. Catching crew required to use hand-washing/sanitisation
4. Catching crew required to use dedicated footwear and fresh clothes.

The enhanced measures reduced the number of *Campylobacter* positives from the hands and shoes of catchers. However, there was no impact on flock colonisation. Regardless of the “enhanced” biosecurity measures, all of the study flocks went positive (26).

A very recent UK study that examined 2,314 poultry batches found that 1/3 of broiler batches that were highly colonised by *Campylobacter* could have been avoided by either enhanced biosecurity or by avoiding thinning (27). A very effective farmer focussed information sheet has been produced as a result of this project (Campy-Biosecurity).

Overall, while biosecurity is always a necessity for the modern poultry production system, there is no realistic hope that a single barrier based on biosecurity can be totally effective.

**Diet:** When xylanase was added to a wheat-based diet, there was a reduced intestinal viscosity and a reduced level of *C. jejuni* colonisation in artificially infected chickens (27). Importantly, the chickens were still colonised but the reduction in *C. jejuni* was statistically significant (27). There appears to have been no follow up published involving field trials in commercial chickens.

US studies have shown that 0.7% caprylic acid in the feed (with or without 12 hour feed withdrawal) consistently resulted in lower caecal *Campylobacter* in market-age broilers (28). The practicality of this approach remains un-tested in field trials.

In a European study, the addition of a commercial fatty acid supplement (Lodestar) resulted 200 times increase in the number of *Campylobacter* cells required to infect 50% of exposed chickens (29).

A very comprehensive European study has recently looked at a range of dietary additives (organic or fatty acids, monoglycerides, plant extracts, prebiotics, or probiotics) and their impact on Campylobacter levels in broiler across the entire rearing period (30). No single treatment was able to prevent *Campylobacter* colonization. However, three compounds (one organic acid, one probiotic and one prebiotic) were able to achieved mean reductions of 2-3 log$_{10}$ in birds that were 42 days old (30).

Overall, dietary manipulations have some potential to reduce the colonisation levels in broilers BUT there is a need for more field based evaluations that look at the performance of these approaches in the real world AND...
the integration of such approaches along with other options (biosecurity and so forth) in a sustainable and cost-effective manner.

**Competitive Exclusion (CE):** A review by Mead (10) concluded that there is little evidence that commercially available CE preparations that are targeted at *Salmonella* control are effective, under field conditions, against campylobacter. Both direct caecal contents (31) or caecal contents that have been cultured under very strict anaerobic conditions (32) have some ability to reduce Campylobacter colonisation. However, both methods have problems in many countries due to regulatory issues about biological products. As well, there are practical production issues about these approaches. Overall, they do not appear fruitful avenues for further work.

**Vaccines:** There are currently no commercial vaccines for the control of Campylobacter in poultry. This is probably due to the difficulty of developing a vaccine against an organism which has great genetic/antigenic diversity and which is essentially a commensal gut organism. Early studies showed that vaccines could have an effect on colonisation BUT not at a biologically significant level as reviewed by Zhang (33).

In recent times, a renewed interest in the Campylobacter vaccines is evident. A group in Poland showed that birds given a live, attenuated *Salmonella* vaccine which expressed an inserted Campylobacter gene had lower caecal Campylobacter colonisation rate than unvaccinated controls (34). A US group used another live, attenuated *Salmonella* vaccine that contained a Campylobacter gene and achieved a Campylobacter colonisation level below detection levels following challenge (35).

In a UK based study, a vaccine consisting of live *Eimeria* parasite expressing an inserted Campylobacter gene resulted in a 10 fold reduction in Campylobacter levels following challenge (36).

The Polish group has recently demonstrated that in ovo vaccination (a common vaccination route for many broiler vaccines) is also possible with several different formulations achieving significant reduction in caecal levels (challenge at 14 days of age and results from 21 and 28 days of age) (37).

None of these vaccine studies have yet progressed to field work so the performance of these candidate vaccines in the real world remains uncertain. There is another key issue – many of these vaccines are recombinant organisms. Hence, use of these particular vaccines needs government, industry and consumers to accept of the use of genetically modified organisms as vaccines in food-producing animals.

**Bacteriophages:** Bacteriophages are viruses that affect bacteria – and this case have an ability to lyse or kill Campylobacter. Essentially, bacteriophages prey on bacteria. The advantage of bacteriophages is that they are quite specific. A bacteriophage that infects/kills *Campylobacter jejuni* will not infect/kill other bacterial species such as *Escherichia coli* or *Salmonella*. This specific predation opens the opportunity to use bacteriophages as a targeted killing mechanism.

The concept of using bacteriophages to reduce the levels of *Campylobacter* in chickens has been actively explored since the middle of the 1990s. A three log reduction in the Campylobacter count occurred when phages were given to chickens (38). However, colonisation could not be prevented by use of the bacteriophage ahead of the Campylobacter challenge (38). There have been a number of similar studies with similar results, although often achieving a lower reduction of Campylobacter, as recently reviewed (39).

A key issue with bacteriophages is the specificity of the phage-bacterial interaction. There is a need to use a cocktail or mix of phages to ensure that a wide coverage of Campylobacter occurs. Using a mix of three phages, treatment via oral gavage or in the feed resulted in a 2 log reduction in the faecal count of both *C. jejuni* and *C. coli* (40).

Another key concern has been the development of phage resistance. However, a recent study has provided a new perspective in this area. This German study showed a permanent reduction in caecal Campylobacter levels in chickens given either a single phage or a four-phage cocktail (41). Importantly, while phage resistance occurred in both groups, there was no impact on Campylobacter reduction (41).

As with vaccines, these promising beginnings have not yet been confirmed by follow-up field studies. These field studies need to address practical issues – how to deliver the phage mix as well as efficacy, cost and whether phage resistance is a concern.

**Conclusions**

A consistent theme in this review of on-farm control of Campylobacter is that no one treatment or strategy is likely to provide effective control. However, the biology of the situation (an organism that only grows in environments that resemble the poultry gut) means that progressive barriers that each achieve only a partial reduction are potentially very effective in terms of public health outcomes. A real question that needs to be addressed is “Who pays for Campylobacter control programs?” This question confronts all food safety programs and has not been sufficiently understood by the regulators, consumers and society in general.
References