EPIDEMIOLOGICAL STUDIES OF CAMPYLOBACTER COLONISATION OF BROILER FLOCKS IN SOUTH EAST QUEENSLAND

J.K. MIFLIN\textsuperscript{1}, J.M. TEMPLETON\textsuperscript{1} and S.J. MORE\textsuperscript{2}

Summary

Detailed epidemiological studies on selected broiler farms were conducted in an attempt to elucidate mechanisms of transmission of \textit{Campylobacter} spp. in poultry. \textit{Campylobacter} colonisation of flocks was not detected prior to 28 d of age. Results to date indicate that drinking water and darkling beetles were not the primary source of introduction to the flock but did play an important role in horizontal transmission. Transport crates used at partial depopulation can introduce \textit{Campylobacter} spp. into a flock. However, with stringent biosecurity on flocks housed in modern tunnel sheds, \textit{C. jejuni} colonisation can be prevented to final slaughter at 47 d of age.

I. INTRODUCTION

\textit{Campylobacter} enteritis is the most common notifiable infection in humans in Australia. The causative bacteria, \textit{C. jejuni} and \textit{C. coli}, cause a spectrum of illness in which diarrhoea is the most common clinical sign. Since \textit{Campylobacter} spp. live as commensals in the intestinal tract of a wide range of birds and mammals there are a number of pathways of human infection. The vast majority of \textit{Campylobacter} cases occur as sporadic infections rather than as large outbreaks. Handling or consumption of raw or undercooked poultry is considered to be the most important risk factor and errors in food handling are also often involved in sporadic cases. The infectious dose in humans is 500-800 bacterial cells.

The optimum growth temperature for \textit{C. jejuni} and \textit{C. coli} is around 42°C. The primary site of colonisation in the chicken is the lower gastrointestinal tract, especially the caeca. During evisceration, campylobacters spill over onto the carcass, and further cross-contamination occurs during the spin-chilling process. Prevalence at the retail level can be very high with overseas studies recording up to 98\% of samples positive (Jacobs-Reitsma, 2000).

The low infectious dose means that reducing the number of flocks that carry \textit{Campylobacter} spp. will have a greater impact on public health than reducing the number of these bacteria on carcasses. It is generally accepted that control strategies should, therefore, be applied at the farm level to prevent colonisation of flocks.

II. METHODS

In order to study the source of \textit{Campylobacter} and also aspects of transmission, a longitudinal study design was adopted. Sampling began at placement and continued at frequent intervals to determine at what age colonisation was first detected and how rapidly the organism spread within the flock. Systematic random sampling was used during faecal sample collection. Faecal samples were plated directly onto Karmali agar and incubated at

\textsuperscript{1} Agency for Food and Fibre Sciences, QLDPI, Animal Research Institute, Yeerongpilly, Queensland 4015.
\textsuperscript{2} School of Veterinary Science, The University of Queensland, Pinjarra Hills, Queensland 4069.
42°C in an atmosphere of 5% O₂, 10% CO₂ and 85% N₂. Sample size (100 faecal samples per shed) was sufficient to detect a single positive sample with 95% confidence when at least 3% of the birds were colonised. Samples of drinkers, litter and darkling beetles and larvae were enriched in Preston broth overnight and then plated onto Karmali agar.

Molecular methods were used to elucidate the epidemiology of *Campylobacter* in broiler flocks. The DNA typing technique adopted was the *flaA* PCR (Nachamkin et al. 1993), which is based on one of the genes encoding the protein in the bacterial flagellum. The method involves PCR amplification of the *flaA* gene followed by restriction enzyme digestion of the amplified product to generate a series of DNA fragments of different sizes. These fragments are sorted according to size on an agarose gel, and the resulting pattern characterises a particular isolate (see Figure 1).

![Figure 1](image)

**Figure 1**  A single *flaA* type present in different samples in one shed

### III. RESULTS

All flocks tested to date have been *Campylobacter*-free to 28 d of age. When *Campylobacter* spp. were detected before partial depopulation, colonisation occurred in a window between 28 and 35 d of age, irrespective of clean-out procedures, breed of bird, or age of donor flock. Once the first positive sample was detected the flock became 100% positive within 4 to 6 d. Table 1 gives an example of how rapidly the organism spreads within a flock. Transmission within the flock can be directly via the faecal-oral route or via contaminated drinkers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0-28</th>
<th>Day 31</th>
<th>Day 34</th>
<th>Day 37</th>
<th>Day 42</th>
<th>Day 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>negative</td>
<td>2/100 +ve</td>
<td>85/100 +ve</td>
<td>100/100 +ve</td>
<td>30/30 +ve</td>
<td>29/30 +ve</td>
</tr>
<tr>
<td>Drinkers</td>
<td>negative</td>
<td>negative</td>
<td>10/12 +ve</td>
<td>10/12 +ve</td>
<td>12/12 +ve</td>
<td>12/12 +ve</td>
</tr>
<tr>
<td>Litter</td>
<td>negative</td>
<td>negative</td>
<td>1/3 +ve</td>
<td>1/3 +ve</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Beetles/larvae</td>
<td>negative</td>
<td>negative</td>
<td>2/4 +ve</td>
<td>negative</td>
<td>1/6 +ve</td>
<td>2/8 +ve</td>
</tr>
</tbody>
</table>

(a) Drinking water

As shown in Table 1, at Day 31, 2/100 faecal samples were positive while all other samples including drinkers were negative. *Campylobacter* spp. were not detected in drinkers

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prior to the chickens becoming positive, suggesting that drinkers were not the source of introduction to the flock.

(b) Darkling beetles and larvae

Litter beetles found at placement were invariably Campylobacter-negative. Furthermore, C. jejuni was only detected in the beetles or larvae after the chickens became colonised. On the basis of results obtained to date, litter beetles did not serve as a reservoir of Campylobacter as they do not maintain the organism between cycles.

(c) Litter/shed environment

To date, C. jejuni has not been isolated from litter prior to colonisation of the chickens. On one of the farms studied, litter was re-used from one batch to the next. In one of the first sheds colonised in the second production cycle on this farm, the flaA type observed was the same type as was present in that shed in the previous batch. This could suggest carryover in the litter or within the shed environment in this shed. The results could also be interpreted to illustrate the existence of a common reservoir that infected this shed in both cycles. A new, different flaA type appeared simultaneously in one of the other study sheds which makes interpretation more complex. Future work will investigate whether wet floors might be involved in this situation.

(d) Farm animals

Campylobacter isolation was attempted from 70 separate samples of cattle faeces from one poultry farm over three production cycles. From these samples, one C. jejuni isolate was obtained from each of two consecutive production cycles. Typing of these two isolates demonstrated that they were identical to each other. However, this was a different flaA type from any of the types seen in the chickens on the farm in any of the three cycles. In this particular instance, there was no evidence of movement of C. jejuni from cattle to poultry, or vice versa.

(e) Vertical transmission

In an experiment to examine whether or not vertical transmission was occurring, 1-d-old chicks from a 56-week old parent breeder flock were placed on two different farms. The breeder flock was unusual in that it was colonised with only one flaA type. On one farm the progeny broilers remained negative throughout the sampling period until final slaughter. On the other farm, the flock was fully colonised by 35 d of age with a distinctly different flaA type from that found in the breeder flock. These results indicate that vertical transmission did not occur in this experiment.

(f) Crates

On one study farm, flaA typing has demonstrated that unwashed transport crates used at partial depopulation introduced Campylobacter spp. into a flock.
(g) Risk reducing measures

The longitudinal studies conducted to date have included one farm with very high standards of biosecurity. The farm has modern tunnel sheds with stabilised concrete floors. Each shed has an entrance room where outside shoes are changed for shed-dedicated footwear. Birds in shed 3, the shed initially selected for detailed study, were negative throughout the growing period, but were slaughtered early at 38 d of age. Detailed monitoring (100 faecal samples per visit) was continued in Shed 1. This shed was thinned at 38 and 42 d of age. All samples were negative till final slaughter at 47 d of age. The results suggest that with stringent biosecurity, Campylobacter spp. can be kept out of poultry flocks.

IV. DISCUSSION

At the commencement of this study, there was little published information on Campylobacter spp. in Australian broiler flocks, apart from two small-scale surveys in the early 1980s (Smeltzer, 1981; Shanker et al., 1982). The results reported above are by no means complete, but are starting to contribute to a local picture that may be used in comparisons with overseas studies. For example, the studies to date have indicated that drinking water is unlikely to be responsible for introduction of Campylobacter to poultry sheds. However, one study in the UK clearly identified drinking water as the source (Pearson et al., 1993) despite the fact that the organism could never be cultured. Furthermore, non-disinfected surface water was shown to be strongly associated with Campylobacter colonisation in Norway (Kapperud et al., 1993).

Despite two decades of concentrated research overseas the sources of infection of poultry flocks are still debatable (Newell and Wagenaar, 2000). Most of the evidence points to horizontal transmission from the environment and the primary strategy employed overseas has been to enhance biosecurity to prevent the entry of the organism into the broiler house. However, it is important to be able to target biosecurity measures to the demonstrated source or sources. Over the next twelve months further studies will be conducted to clarify the source or sources of the organism and to determine the most appropriate on-farm control measures.

REFERENCES


