On-farm Campylobacter and Escherichia coli in commercial broiler chickens: Re-used bedding does not influence Campylobacter emergence and levels across sequential farming cycles

H. N. Chinivasagam, W. Estella, H. Rodrigues, D. G. Mayer, C. Weyand, T. Tran, A. Onysk, and I. Diallo

ABSTRACT  Limitations in quality bedding material have resulted in the growing need to re-use litter during broiler farming in some countries, which can be of concern from a food-safety perspective. The aim of this study was to compare the Campylobacter levels in ceca and litter across three litter treatments under commercial farming conditions. The litter treatments were (a) the use of new litter after each farming cycle; (b) an Australian partial litter re-use practice; and (c) a full litter re-use practice. The study was carried out on two farms over two years (Farm 1, from 2009–2010 and Farm 2, from 2010–2011), across three sheds (35,000 to 40,000 chickens/shed) on each farm, adopting three different litter treatments across six commercial cycles. A random sampling design was adopted to test litter and ceca for Campylobacter and Escherichia coli, prior to commercial first thin-out and final pick-up. Campylobacter levels varied little across litter practices and farming cycles on each farm and were in the range of log 8.0–9.0 CFU/g in ceca and log 4.0–6.0 MPN/g for litter. Similarly the E. coli in ceca were ∼log 7.0 CFU/g. At first thin-out and final pick-up, the statistical analysis for both litter and ceca showed that the three-way interaction (treatments by farms by times) was highly significant (P < 0.01), indicating that the patterns of Campylobacter emergence/presence across time vary between the farms, cycles and pickups. The emergence and levels of both organisms were not influenced by litter treatments across the six farming cycles on both farms. Either C. jejuni or C. coli could be the dominant species across litter and ceca, and this phenomenon could not be attributed to specific litter treatments. Irrespective of the litter treatments in place, cycle 2 on Farm 2 remained Campylobacter-free. These outcomes suggest that litter treatments did not directly influence the time of emergence and levels of Campylobacter and E. coli during commercial farming.

Key words: Campylobacter, E. coli, litter, ceca, chickens

INTRODUCTION

Campylobacter is the common cause of human infectious intestinal disease, mostly in temperate countries (Alter and Scherer, 2006), and is closely associated with poultry and contaminated poultry meat (Wassenaar, 2011). The on-farm management of Campylobacter continues to remain a challenge. Flocks with a higher prevalence of Campylobacter can have an average of log 5.3 colony forming units (CFU) per carcass (and maximum of log 8.0 CFU per carcass) (Allen et al., 2007); thus, they can be of food-safety concern. C. jejuni is the most common species associated with human illness and has evolved to preferentially colonize the chicken gut (Snelling et al., 2005). The ecology and the epidemiology of Campylobacter in broiler flocks is complex (Hermans et al., 2012). The biology of Campylobacter differs from other zoonotic pathogens (such as Salmonella), and there exists a need for a better understanding of Campylobacter physiology and survival mechanisms in poultry (Ingmer, 2011). These aspects can be directly impacted by both the farming environment and practices adopted across various countries.

The expansion of the poultry industry, along with the scale of production, demands continuous supplies of large volumes of quality bedding material (e.g., wood
shavings). There are also related environmental challenges linked to the disposal of bedding after a relatively short poultry farming cycle of approximately 50 d or less where used litter cannot remain on-farm due to biosecurity requirements. These demands have contributed to litter re-use in countries such as Australia and the United States. The emerging practice of re-using bedding (or litter) may have an impact on key food-safety pathogens such as Campylobacter. In the US, litter can be re-used for periods lasting for up to 1 to 2 years or more before a full clean-out of litter from a shed (or barn) occurs (Payne et al., 2006; LSU Ag Center, 2011). In Australia, litter can remain in a shed for more than a single farming cycle and up to several cycles (Chinivasagam, 2009). Thus, the prospect of safely re-using litter can contribute towards alleviating food-safety concerns due to the ongoing challenge of managing Campylobacter during commercial farming.

On-farm biosecurity during poultry farming is an integral part of farming practice targeting the management of pathogens. Thus, studies have looked at possible biosecurity-based interventions and strategies to reduce on-farm Campylobacter (Newell et al., 2011), though it is reported that simple measures do not have a significant influence on the Campylobacter status of the flock (Nather et al., 2009). Thus, common hygienic measures that have substantially reduced Salmonella have not been effective against Campylobacter (Ingmer, 2011). An understanding of the on-farm microbial ecology of pathogens such as Campylobacter (Jaykus, 2003) is a key factor to the acceptance of litter re-use, which can be viewed as a concern during commercial farming.

The cecum is the main source for Campylobacter colonization (Van Deun et al., 2008), and intestinal microbiota perform an important role in controlling enteric bacterial pathogens (Chambers and Gong, 2011). An Australian study (Torok et al., 2009) has shown that the cecal microbiota of chickens raised on reused litter was significantly \( (P < 0.05) \) different from that of chickens raised on any of the other litter materials. This study has also shown the influence of bird age with cecal microbial communities (Torok et al., 2009). Chickens are known to ingest litter (which also plays a role in digestion) (Hetland et al., 2003), and studies have demonstrated a significant consumption of litter among broilers from the floor (Svihus et al., 2009). There could be a relationship between the litter practices adopted and the Campylobacter numbers in the cecum. Campylobacter has shown to have a low infectious dose (approximately 500 organisms) (Robinson, 1981), and thus the management of Campylobacter numbers (in ceca) on-farm can contribute to enhanced product safety.

The main aim of the current study was to assess the levels of Campylobacter (and E. coli) in both litter and ceca in the presence of litter re-use (and without) during normal commercial farming. Three different litter treatments; the conventional practice of cleaning litter after each farming cycle (and using new bedding), the Australian practice of partial re-use (with a mix of used and new bedding) and a full litter re-use practice were studied. In order to assess the impact over time, the three litter treatments were assessed across six sequential farming cycles on two farms.

**MATERIALS AND METHODS**

**Farm Sampling**

Two separate commercial farms, situated close to Brisbane, Queensland (Australia) with a history of litter re-use (over 20 years) were selected. Farm 1 (F1) had 4 sheds with approximately 35,000 chickens per shed, and Farm 2 (F2) had 8 sheds with approximately 40,000 chickens per shed. Only three sheds from each farm were included in this study. The farms were adjacent to each other and were managed by a single company. All normal farming practices, including diet, were according to standard industry practice for meat-chicken rearing, as the trial was carried out under commercial farming conditions. The details of the Australian litter re-use practice adopted by commercial farms is described in Chinivasagam et al. (2012), and the litter management practices that occur between farming cycles are described in Chinivasagam (2009).

Animal ethics approval was obtained for the entire experimental study from the Department of Agriculture and Fisheries (Queensland, Australia) animal ethics committee prior to the trial. Prior to the commencement of the trial, three adjacent sheds from each farm underwent a full clean-out of litter, and that cycle (time taken from chick placement to final pick-up) was designated as cycle 1 (Cy1); hence all three sheds had similar litter treatments during Cy1. From cycle 2 (Cy2) onwards, each shed on both farms had different litter treatments. The litter treatments adopted were designated as “New” (N), “Partial re-use” (P) and “Full Re-use” (F); the conditions adopted on-farm during the trial are described in Table 1. The duration of the six sequential farming cycles were: F1, 23/03/2009 – 13/04/2010 and F2, 29/09/2010 – 09/09/2011.

**Collection of Litter and Ceca Samples**

During each cycle both litter (L) and chickens’ ceca (C) were sampled from the three sheds on both farms as follows: (a) at ~7 days into the growth cycle, litter only, (data not shown); (b) just before first pick-up (thinning), litter and chickens; (c) just before final pick-up, litter and chickens. Pick-ups were commercial pick-up days. Litter was collected as in Chinivasagam et al. (2012) to a depth of 40 cm over an area of 400 cm² using a specially designed stainless steel sampler. The sheds were around 100 m long with 39 (F1) and 33 (F2) bays (structure supports along the shed), respectively, which were used as markers for random number allocation (and sample collection) across the
shed. Each shed was categorized into four main sections, i.e., two in the brooder area (front-end, 1 and 2) and two in the grow-out area (back-end, 3 and 4).

Sample collection was based on the location of bays, drinkers and feeder markings for random number allocation for sampling spots. Initially sixteen bays were selected using random number (i.e., four bays per section). Within a selected bay, eight spots (samples) were selected from an area adjacent to a drinker and/or feeder area, and/or from the centre of the shed which were also selected via random numbers. These eight samples were mixed, and quartered to form a single composited sample for the relevant section, e.g., brooder 1. The same was done for brooder 2, grow-out 1, and grow-out 2, resulting in four main samples per shed (from a total of 32 litter samples per shed), representing the four main sections. Two chickens were collected in the region of the bays selected for sampling of litter resulting in eight chickens per section (e.g., brooder 1). These eight chickens were aseptically dissected on site to remove the ceca, which were then composited to form a single sample for that section (e.g., brooder 1); thus, a total of 32 chickens (samples) were collected per shed representing the four main sections. Final composited samples of litter and chicken were stored chilled. Litter was sampled on arrival at the laboratory; ceca were sampled within 22 hours of sample collection. Litter pH was measured as in Chinivasagam et al. (2012).

Enumeration and Characterization of Campylobacter

Twenty-five grams of litter or ceca were weighed into 225 mL of Preston broth without antibiotics (Nutrient broth No2 with 5% lysis horse blood). The ceca were stomached (Smasher AESAPI064) and the litter was blended using a stick blender (barmix) for 1 minute. Campylobacter levels in litter were determined using a three-tube Most Probable Number (MPN) method as described by Chinivasagam et al. (2009). Serial dilutions of ceca were directly plated onto CCDA with selective supplement (Oxoid SR0155) and incubated at 37°C for 48 h under micro-aerobic conditions using Campygen (Oxoid, CN0025A). For both litter and ceca, three to five typical greyish, shiny, moist colonies representative of Campylobacter were randomly picked from the appropriate or countable CCDA plate and streaked onto Abeyta-Hunt-Bark agar as in Chinivasagam et al. (2009) for confirmation. DNA extracts were prepared by re-suspending a loopful of growth in 100 μl of sterile distilled water and heating at 95°C for 30 min. The suspension was cooled, centrifuged (at 14,000 g for 45 s) and stored (at −18°C). The isolates were confirmed as Campylobacter spp. by a real-time PCR (Best et al., 2003) following which Campylobacter levels were presented as MPN/g for litter and CFU/g for ceca. The minimum detection for litter was log 0.6 MPN/g and for ceca log 2.0 CFU/g. The Campylobacter isolates were confirmed as C. jejuni or C. coli using the real-time PCR described by Best et al. (2003). A total of 825 isolates, 611 from F2 and 214 from F1, were positively identified to the species level and were presented as percentage species distribution. E. coli levels were enumerated as described in Chinivasagam et al., 2009 using the same serial dilutions used for litter and ceca.

Statistical Methodology

Prior to commencement of the trial, “shed effects” sampling of surface litter was carried out, comparing the three sheds on F1. Analysis of variance for water activity, moisture, pH, and temperature showed no notable impact of the shed environment (data not presented). The study sheds were found to be approximately equal, thus validating the study design adopted on F1 and F2.

The time-series nature of the data was taken into account by an analysis of variance of repeated measures (Rowell and Walters, 1976), via the AREPMEASURES

| Nature of bedding in sheds during the study on F1 and F2 | New Shed 1 | Partial Re-use Shed 2 | Full Re-use Shed 3 |
|-------------------------------------------------------|------------|----------------------|-------------------|
| Always new.                                           | (1)        | (1)                  | (1)               |
| Litter removed after each cycle and new bedding placed.| (2)        | (2)                  | (2)               |

| Litter management between cycles during the study on F1 and F2 (all sheds cleaned sanitized between cycles, sprayed for insects) | New bedding placed across whole shed | Litter from brooder heaped into pile at grow-out end for ∼5 to 6 d and then spread at grow-out end. Clumps removed. | (1) Farm 1: Litter heaped across entire length of shed for 5 d and spread. (2) Farm 2: Litter aerated mechanically, clumps removed and spread. |

| Biosecurity measures during cycles on F1 and F2 | The use of footbaths with disinfectant and protective clothing on entry of authorized personnel |
|------------------------------------------------|-----------------------------------------------------------------------------------------------|

#Sixth cycle was run as free-range practice (Free Range Egg & Poultry Australia - FREPA) due to conversion of that farm to free-range.

##Described in (Chinivasagam et al., 2012 and Chinivasagam 2009).
procedure of (GenStat, 2013). This forms an approximate split-plot analysis of variance (split for time). The Greenhouse-Geisser epsilon estimates the degree of temporal autocorrelation, and adjusts the probability levels for this. Sheds (within sites) were taken as the experimental unit, as the litter treatments were applied at this level. End of shed (brooder/growing) was included as a split-plot design within sheds, with a second split in the analysis of variance for times. Observations recorded as ‘less than limit of detection’ (LoD) were included in the analyses as half the LoD. As the sites had differing management practices, sites were included as a factor of interest (rather than as a random or blocking effect). The 5% significance level ($P = 0.05$) is used throughout. The four-way interaction (sites by treatments by shed-ends by times) was not significant in all analyses, so was dropped from the model.

**RESULTS**

At the commencement of each cycle, sampling for litter only was carried out on d 7 to 9 (data not shown). During the 2-year period of this trial across farms cycles and treatments, *Campylobacter* was detected in litter only once, on d 9, cycle 6, F1, in “new litter treatment” and only at low levels, log 1.0–3.0 MPN/g (i.e., in both brooder-end segments only, where the chicks were present) and not in the chicken-free grow-out end of the shed. The farm was run as free-range for the first time (Table 1), though the flock was never out on the range during that period. Thus, other than this single and low-level instance, *Campylobacter* was never detected early in any cycle on both farms.

At first thin-out and final pick-up (analyzed separately), the statistical analysis for both litter and ceca showed that the three-way interaction (treatments by farms by times) was highly significant ($P < 0.01$), indicating that the patterns of *Campylobacter* emergence/presence across time vary between the farms, treatment, cycles, and pick-ups. These differences over cycles and time were not related to the litter treatments even though the litter treatments remained the same across two independent farms. *Campylobacter* levels in ceca and litter across six farming cycles on both farms for the three litter treatments are presented in Table 2.

### Campylobacter, First Thin-Out: Ceca

First thin-out ranged from d 26 to 35 across both farms (Table 2). During Cy1, the three litter treatments were similar (all-new bedding) as it followed a full clean-out of litter across the three test sheds on each farms. Irrespective of this situation, during Cy1 on F2, *Campylobacter* was detected only in the shed allocated to partial re-use, (ceca, log 8.5 CFU/g). Similarly during Cy1 on F1, *Campylobacter* was detected

---

**Table 2. Campylobacter** levels in ceca* (C) (log CFU/g) and litter* (L) (log MPN/g) during first thin-out and final pick-up on Farms 1 (F1, 2009–2010) and Farm 2 (F2, 2010–2011) in new, partial and full re-use litter treatments across six farming cycles (Cy1, Cy2, Cy3, Cy4, Cy5, Cy6).

---

|       | Cy1** | Cy2 | Cy3 | Cy4 | Cy5 | Cy6 |
|-------|-------|-----|-----|-----|-----|-----|
| **CECA** |       |     |     |     |     |     |
| **Day** | F1    | F2  | F1  | F2  | F1  | F2  |
| New    | nd    | nd  | nd  | nd  | nd  | nd  |
| Partial| 2.5   | 8.5 | nd  | nd  | nd  | nd  |
| Full   | 2.8   | nd  | nd  | nd  | nd  | nd  |
| **LITTER** | F1  | F2  | F1  | F2  | F1  | F2  |
| New    | nd    | nd  | nd  | nd  | nd  | nd  |
| Partial| 2.9   | nd  | nd  | nd  | nd  | nd  |
| Full   | nd    | nd  | nd  | nd  | nd  | nd  |
| **Final pick-up** | F1 | F2  | F1  | F2  | F1  | F2  |
| New    | 6.8   | 7.2 | 8.1 | nd  | 8.3 | 8.5 |
| Partial| 6.7   | 9.0 | 8.9 | nd  | 8.3 | 8.5 |
| Full   | 6.4   | 8.7 | 8.9 | nd  | 7.5 | 7.5 |

---

*Mean of C1, C2, from brooder end and C3, C4 from grow-out end of a shed of allocated treatment.

*Mean of L1, L2 from brooder end and L3, L4 from the grow out end of shed of allocated treatment.

**All treatments equal during C1 (cycle 1).

*During cycle 5 Farm 1 was picked up early.

^All three litter treatments were in place but run as a free range cycle.

nd, not detected (minimum detection - litter log 0.6 MPN/g and ceca log 2 CFU/g).

Within farms, cycles and pick-ups - treatment means with different superscripts are significantly ($P < 0.05$) different.
in ceca (log 2.5 and 2.8 CFU/g) in the two sheds allocated to both re-use treatments but not detected in new litter. Thus, there were differences in the pattern of emergence after a full clean-out of litter (Table 2).

Subsequently on F1, through four sequential cycles and until Cy6, Campylobacter did not emerge across all three litter treatments (not detected in litter and ceca). This was in contrast to F2, when Campylobacter emerged either intermittently (Cy3 and 5) or throughout litter treatments (Cy4 and 6) during the rest of the trial (Table 2). More specifically on F1 (Cy6), Campylobacter was detected in ceca at high levels (log 9.4 and log 9.3 CFU/g) in both new and partial re-use but not in full re-use; the levels were not significantly different. When Campylobacter was present intermittently on F2 (Cy3), it was present only in new litter treatment (log 4.8 CFU/g), while during Cy5, it was present in both partial (log 6.0 CFU/g) and full re-use (log 7.3 CFU/g) litter treatments; these levels were not significantly different. In contrast, when Campylobacter was present throughout all three litter treatments, on F2 (Cy4), the levels (in ceca) were also not significantly different (ranged log 8.0–8.6 CFU/g). On F2 (Cy6) Campylobacter was present across all litter treatments and both partial re-use (log 8.8 CFU/g) and full re-use (log 8.0 CFU/g) were not significantly different compared to the new (log 7.2 CFU/g, P < 0.05). Thus from an overall perspective the pattern of Campylobacter emergence (and levels) on both farms during first thin-out across each cycle did not show any consistent pattern attributed to the litter treatments.

**Campylobacter, Final Pick-up - Ceca**

Final pick-up ranged from d 45 to 51 (F1 and F2), with the exception of early pick-up on F1 (Cy5 and Cy6) Table 2. Just prior to final pick-up, Campylobacter had emerged across the majority of the litter treatments and was generally present at high levels (log 7.5–9.0 CFU/g) on both farms with the exception of F1, Cy1 (log 6.4–6.8 CFU/g across all three sheds), Table 2. On F1, Campylobacter levels in ceca during cycles 2, 3, 4, and 6 were not significantly different (P < 0.05) across the three litter treatments and were also in the similar range, (Table 2). The final cycle (Cy6) on F1 was run as a free-range cycle with high Campylobacter levels (log 9.0, 8.9, 9.0 CFU/g) the levels not significantly different, (Table 2). It was interesting to note that on F2, Cy2 remained a “Campylobacter-free cycle” across all three litter treatments (during both final pick-up and thin-out) with the organism never detected in either ceca or litter. Campylobacter was present across the rest of the cycles on F2, with levels during cycles 5 and 6 not significantly different (i.e., Cy5 log 8.6 to log 8.8 CFU/g and Cy6 log 8.5 to log 8.9 CFU/g) across all litter treatments (Table 2).

Thus, among the total of 10 cycles across both farms (total 12 but exclusive of Cy1), Campylobacter levels were significantly different (P < 0.05) across litter treatments only three times (two from F2 and one from F1), (Table 2). On F1, Cy5 low Campylobacter levels were detected prior to final pick-up only in new litter treatment (log 4.6) and below detection in both partial and full re-use litter treatments which can be attributed to an earlier than normal commercial final pick-up (d 41). Thus, as with the results for first thin-out, during final pick-up the differences between litter treatments were not related to the influence of any particular litter treatment, across both cycles and farms.

**Campylobacter, First Thin-Out and Final Pick-Up: Litter**

Campylobacter levels in litter were generally four logs lower than the levels in ceca and the presence/absence of the organism in litter was always linked to ceca except on one occasion (Table 2). On F1, Cy1 the cecal Campylobacter levels were only low (log 2.5–2.8 MPN/g), a possible reason to be non-detectable in litter during first thin-out. The litter – ceca link could also be further demonstrated during Cy2, on F2, the “Campylobacter-free” cycle when the organism was not detected in litter nor in ceca. When comparing litter treatments and cycles, Campylobacter levels on F1 were not significantly different between litter treatments during three cycles (Cy1, 2, 6) and four sequential cycles (Cy1, 2, 3, 4) on F2 during final pick-up.

Interestingly, as the cycles on F2 progressed and during Cy5 (final pick-up), there tended to be a reduction in Campylobacter levels in full re-use litter (log 3.0 MPN/g) that was significantly lower (different P < 0.05) than both new (log 5.5 MPN/g) and partial re-use litter (log 4.9 MPN/g) Table 2. The reduction in full re-use continued to be observed on F2 (Cy6) though the Campylobacter levels in full re-use litter (log 4.2 MPN/g), were not significantly different to partial re-use (log 5.2 MPN/g) but significantly different to new (log 5.8 MPN/g, P < 0.05) Table 2. Irrespective of this reduction in Campylobacter levels in full-reuse litter, this pattern was not reflected in the ceca during those two cycles, suggesting that the litter treatment was not having an impact on Campylobacter levels in ceca.

On F1, two cycles (Cy3 and 4) presented variations across litter treatments during final pick-up, but these variations were not uniform. For example during Cy3, Campylobacter levels in both new (log 2.7 MPN/g) and partial re-use (log 2.2 MPN/g) were significantly different to full re-use litter (log 4.8 MPN/g, P < 0.05). A similar situation was observed during Cy4, F1, with the exception that full-re-use was not significantly different to new litter. As was for ceca, Cy5, F1 was the only cycle during which Campylobacter was not detected across two litter treatments (new and full re-use) which could be attributed to the early final pick-up on that farm at d 41, (Table 2).
Table 3. *E. coli* levels in ceca* (C) (log CFU/g) during first thin-out and final pick-up on Farms 1 (F1, 2009–2010) and Farm 2 (F2, 2010–2011) in new, partial and full re-use litter treatments across six farming cycles (Cy1, Cy2, Cy3, Cy4, Cy5, Cy6).

|                | First thin-out | Final pick-up |
|----------------|---------------|---------------|
|                | Cy1** Cy2 Cy3 Cy4 Cy5 Cy6 |              |
| day            | 27 28 28 28 28 28 | 51 48 49 51 50 49 |
| New            | F1 F2 F1 F2 F1 F2 | F1 F2 F1 F2 F1 F2 |
| Partial        | 8.3 8.3 8.3 7.9 8.1 7.8 | 7.5 7.9 7.5 7.9 7.5 7.9 |
| Full           | 8.1 7.9 7.9 8.0 8.1 8.0 | 7.3 7.2 7.3 7.3 7.3 7.3 |

Table 4. Overall main effects of the litter treatments (i.e., averaged across farms and times).

|                | P-level for litter trt. | Full | New | Partial | Standard error |
|----------------|-------------------------|------|-----|---------|----------------|
| Campylobacter in ceca | 0.46                    | 5.15 | 5.43 | 5.90    | 0.349          |
| Campylobacter in litter | 0.11                    | 2.30 | 2.60 | 2.58    | 0.057          |
| *E. coli* in ceca     | 0.13                    | 7.73 | 7.77 | 7.61    | 0.032          |

Table 5. Percentage* Campylobacter species distribution across *cycles 1 through 6 (except 5) on Farm 1.

|                | C. jejuni | C. coli |
|----------------|-----------|---------|
| Cycle 1        | 100       | 0       |
| Cycle 2        | 84        | 16      |
| Cycle 3        | 63        | 37      |
| Cycle 4        | 28        | 72      |
| Cycle 6        | 16        | 84      |
| Total isolates | 201       |         |

*Combined litter and ceca for new, partial and full re-use treatments.

that, compared to the degree of variation within each shed, the six individual sheds are effectively following different time-paths (both within and across cycles, and between the farms). The overall main effects of the litter treatments (i.e., averaged across farms and times) were not significant, as shown in Table 4.

**E. coli, First Thin-Out and Final Pick-Up: Ceca**

Table 3 lists the mean *E. coli* levels in ceca. *E. coli* levels in both litter (not presented) and ceca did not vary much across treatments and cycles. In litter the highest *E. coli* levels (log 8.0 CFU/g) were generally evident around d 7 (data not shown) at the brooder ends, when young chicks are present. Unlike *Campylobacter*, *E. coli* was widely present across both thin-out and final pick-up, with not much variation between levels. From an overall perspective *E. coli* levels remained high and ranged from log 7.0–8.0 CFU/g. Just prior to first thin-out, there were no significant differences in *E. coli* levels in ceca between litter treatments across five cycles on both F1 (Cy1, 3, 4, 5, 6) and F2 (Cy1, 2, 4, 5, 6). Similarly just prior to final pick-up, *E. coli* levels in ceca were not significantly different between litter treatments across five cycles (Cy 1, 3, 4, 5, 6) on F1 and four cycles on F2 (Cy1, 2, 3, 4). There were some significant differences in Cy2 and 3. On F1 (Cy2) *E. coli* levels of both full re-use (log 7.9 CFU/g) and new (log 8.2 CFU/g) were significantly different (P < 0.05) from partial re-use (log 6.5 CFU/g). In contrast, during Cy3 on F2 partial (log 7.9 CFU/g) and full re-use (log 8.0 CFU/g) levels were significantly lower than in new (log 8.6 CFU/g, P < 0.05).

**Overall Effects of Litter Treatments on Campylobacter and E. coli**

Tables 2 and 3 show varying patterns over time for the responses to the litter treatments. This indicates

**Campylobacter Species Dominance Across Cycles**

Table 5 presents a summary of the *Campylobacter* species diversity combined across litter, ceca, and treatments for each cycle on F1. During cycle 1, *C. jejuni* was dominant (100%) with a gradual transition to *C. coli* as the cycles progressed towards the sixth cycle (84%). More detailed analysis of patterns on F2 (Figure 1) illustrates the percentage of *C. jejuni* or *C. coli* in litter and ceca across treatments (new, partial, full) and cycles prior to first thin-out and prior to pick-up. *C. coli*
**DISCUSSION**

There is an intimate relationship between the bird and its bedding due to the potential for chickens to ingest litter during the farming cycle. Due to bird excretion, litter can be a source of microbiota, including *Campylobacter*, and this can influence the colonization and development of cecal microbiota (Torok et al., 2009). The reciprocal effects between the microbiotas tended to be associated with ceca in e.g., Cy1, 5, 6, and *C. jejuni* tended to be associated with litter (e.g., Cy3, 4, 6). There were tendencies for change in species mix between first thin-out and final pick-up (e.g., *C. coli* to *C. jejuni* in litter; Cy3, new; Cy5, partial; Cy6 partial) and *C. jejuni* to *C. coli* in ceca (Cy4, partial; Cy5, full). Thus there was no clear pattern attributable to the litter treatments (and farms), with some cycles totally dominated by one single species both in litter and ceca (Cy4, *C. jejuni* and Cy5, *C. coli*).

**pH Levels in Litter**

The litter pH values were around 8.00 or above close to both pick-up events (Table 6). When comparing the full re-use litter treatment (which had more aged litter) with new (across both pick-ups) there were only five instances when the pH values of full re-use litter were significantly different to new (*P* < 0.05) and only one instance when partial re-use was significantly different to new (*P* < 0.05). These results demonstrate no great variations in litter pH across treatments.
present in the litter and the intestines of broilers can result in fresh litter having more environmental bacteria and re-used litter having bacteria of intestinal origin (Cressman et al., 2010). More specifically, litter has shown to be a source of C. jejuni infection to artificially inoculated chicks reared under controlled conditions (Montrose et al., 1985). Alternatively, the lack of pathogens in re-used litter during farming has been attributed to the presence of flora that is actively involved in the composting of organic matter (Lu et al., 2003b). The present study has shown that on F2, as full-reuse litter aged with time, Campylobacter levels (in litter) were more than two log lower at times (range log 1.6–2.6 MPN/g) than in new and partial re-use litter.

The current study that lasted two years with two farms adopting three different litter treatments across six sequential farming cycles has shown that adopted litter treatments had no relationship with both the emergence and levels of Campylobacter and E. coli in the ceca. However, both Campylobacter and E. coli were widespread in litter and ceca right across the trial; a litter–ceca relationship was apparent also observed for litter and aerosols in commercial broiler sheds (Chinivasagam et al., 2009). E. coli appeared at a very early age of the bird and at high levels until final pick-up. In contrast, Campylobacter emerged more or less at a more mature age of the bird with high levels (similar to E. coli) across the litter treatments.

E. coli was always present both in litter and ceca on both farms across time irrespective of the different litter treatments. High levels of bacteria (1.5 × 10^6 CFU/g) have been isolated from the ceca of newly hatched chicks (up to 21 hours) even when no food was consumed (Shapiro and Sarles, 1949). However, as soon as the chicks consumed food, the coliform levels (all confirmed to be E. coli) increased up to 1.5 × 10^8 CFU/g within a few hours. These levels were maintained at 10^5–10^8 CFU/g from 0–200 d of age (Shapiro and Sarles, 1949) demonstrating the close link between E. coli with the bird right from the start. The ceca of chicken are also associated with various other microbes and can contain up to 10^{10}–10^{11} cells/g of microorganisms within the cecal digesta (Gong et al., 2002). Thus the high levels of E. coli in litter in the present study are more a feature of bird excretion.

In the present study the levels of E. coli (for all the three litter treatments) were approximately log 7.0 CFU/g and ∼log 6.0 CFU/g in ceca and litter respectively with the cecal levels generally being a log higher than in litter. One of the key observations on both farms was the simultaneous uniform distribution of E. coli in both ceca and litter across the brooder and grow-out end sections (i.e., sections 1–4) throughout the six cycles for all three litter treatments. The uniform E. coli levels across brooder and grow-out sections appear to be a result of bird excretion and not as result of the build-up of E. coli levels across sequential re-use cycles where litter is treated by pile-up between re-use. E. coli is widely distributed within the shed (litter and aerosols) (Chinivasagam et al., 2009) and piling of litter between farming cycles has been shown to kill E. coli (Chinivasagam, 2009). It was therefore not surprising to observe in the current study that on d 7, E. coli was either absent or detected only in low levels in spread litter within the chicken-free brooding area (data not shown).

Unlike E. coli, Campylobacter was detected later in the production cycle of all commercial broiler batches monitored on both farms (except cycle 2 on F2 which was “Campylobacter free”). There was only one occasion where Campylobacter was detected at a very early stage of the production cycle, at d 9 in a free-range shed cycle of F1 with new litter and only in the both segments of the brooder end of that shed and (not the chicken-free grow-out end). Where both litter and ceca were collected from the same shed at the same time, litter was always shown to be positive when ceca was positive. Thus, even if the ceca was not tested at this stage, it may be possible that the chickens were Campylobacter-positive at the time. It is not possible that the free-range cycle was contributory, as the chicks did not have had access to the range. It also is unlikely that Campylobacter appearance was a carryover from a previous cycle, as it was not detected in spread litter following pile-up in the chicken-free brooder end at d 9 (data not shown). The organism has been shown to die-off in piled litter between partial re-use cycles (Chinivasagam, 2009). While the exact reason is uncertain, Cox et al. (2012) comprehensively reviewed the possibilities that can contribute to the early emergence

### Table 6. pH levels in litter across six farming cycles for new, partial, and full re-use on Farm A.

|          | Cy1** | Cy2  | Cy3   | Cy4   | Cy5   | Cy6   |
|----------|-------|------|-------|-------|-------|-------|
| **First pick-up** |       |      |       |       |       |       |
| New      | 8.54  | 8.14b| 8.34  | 7.90  | 8.24b | 8.36a |
| Partial  | 8.46  | 8.29b| 8.40  | 8.47  | 8.40b | 8.35a |
| Full     | 8.42  | 8.44a| 8.35  | 8.39  | 8.54a | 8.08b |
| **Final pick-up** |       |      |       |       |       |       |
| New      | 8.52  | 8.34 | 7.70b | 8.25b | 8.64  | 8.25b |
| Partial  | 8.40  | 8.35 | 7.93^a| 8.35^a| 8.40  | 8.76^a|
| Full     | 8.54  | 8.50 | 7.97^a| 8.52^a| 8.47  | 8.59^a|

**All treatments equal during C1 (cycle 1).

^a,bWithin farms, cycles and pickups - treatment means with different superscripts are significantly (P < 0.05) different.
of the organism, and thus it may not be unusual for flocks to be Campylobacter-positive at this early age, although vertical transmission of the organism remains controversial (Cox et al., 2012).

One of the interesting observations in the present study is the striking similarity of “uniform high levels” of Campylobacter and E. coli in the ceca (log 7.0 to log 8.0 CFU/g), and the uniform pattern of their distribution in both farms across the sheds brooder and grow-out areas (sections 1–4), litter treatments and cycles. Campylobacter and E. coli can be associated with the bird at different stages of the bird’s life cycle. Campylobacter a commensal in poultry (Park, 2002), rapidly replicates in the mucus lining of cecal epithelial cells (Van Deun et al., 2008) and is closely associated with the intestinal mucosa and the crypts of the intestinal epithelium (Park, 2002), as E. coli which is associated with the cecal mucosa (Gong et al., 2002). During the present set of trials the cecal levels of Campylobacter were around log 8.0 CFU/g or even higher at times and has been previously reported (Daczkwowska-Kozon et al., 2010). Chicken microbiota plays a major role in the “colonization resistance” of bacterial “pathogens” in the chicken gut (Chambers and Gong, 2011). The succession of gut microflora varies with bird age (Barnes, 1972; Apajalahti et al., 2001) possibly contributing to Campylobacter emerging mid-cycle and E. coli very early in the cycle.

The fact that Campylobacter assumed dominance at almost similar periods (prior both pick-ups) of the bird age (and similar levels) across 11 of the 12 cycles tested may suggest this pattern of dominance may be linked with the microbial succession (or ecology) prevalent in the ceca at the time. A succession of cecal microbial communities from one of transient flora to flora of increased complexity has been shown with bird age (i.e., d 14-28 and 49) (Lu et al., 2003a). Factors such as diet changes (i.e., starter, grower, finisher, and withdrawal) that occur routinely in commercial broilers, can influence the cecal bacterial community structure at various points of the cycle (Apajalahti et al., 2001) which in turn can contribute to Campylobacter proliferation. Thus external risk factors such as first pick-up or origin from sources external to the shed (Allen et al., 2008) seem not to be major drivers of the pattern and emergence of Campylobacter as observed in the current study.

During the present study there was a single cycle (cycle 2) on F2 that was “Campylobacter free” during the entire cycle across all three different litter treatments (three different sheds). This situation seemed unusual and interesting from this perspective that the rest of the five cycles on this farm had appreciable levels of Campylobacter prior to final pick-up across all three litter treatments and nothing varied during cycle 2. While several factors are linked as sources for flock colonization (Bull et al., 2006), there are limited studies on the reasons for a lack of colonization of a flock. Factors such as diet (Fernandez et al., 2000) immune status of the host and environmental conditions in the production system are said to play a role in Campylobacter colonization (Sahin et al., 2002). On-farm biosecurity (Allen et al., 2011) can prevent Campylobacter colonization though biosecurity was already an integral part of the current trial across all the rest of the cycles on both farms as was in cycle 2.

While the colonization dose may vary, the dose for colonization of 50% of the flock (or CD50) has been estimated to be 524 CFU. Once infected, Campylobacter can transmit via coprophagia (Line et al., 2008) and subsequent transmission rates within the shed will vary according to the commercial farming conditions and/or differing litter treatments. While innate immune responses can alter the host microbe interaction and has been attributed to colonization resistance between both resistant and susceptible birds (Connell et al., 2012) this alone does not fully explain why three sheds with different litter treatments were Campylobacter negative during an entire cycle of 51 d. The Cobb birds present across the three Campylobacter-free sheds during cycle 2 were from multiple parent stock without any clear link to the status of the parent flocks. A recent study by Gormley et al. (2014) has shown that both the chicken growth rate and breed did not contribute to a higher risk. These outcomes further emphasize the complexities involved in understanding Campylobacter colonization of flock, an area that requires further research.

Both C. jejuni and C. coli were widely distributed across both cycles and farms but their pattern of distribution could not be attributed to any litter treatment. On F1 there was a gradual transition of C. jejuni to C. coli (ceca and litter combined) across the three treatments and cycles. On F2 a more detailed analysis between litter and ceca presented a more complex pattern with both species represented. In the majority of the instances there were differences between litter and ceca but on other instances the species diversity matched. The disappearance of C. jejuni in organic flock coincided with the appearance of both bacteriophages and C. coli as the dominant strain (El-Shibiny et al., 2005). Host immunity or host mediated changes in the gut flora have also been suggested as possible contributory factors for initial C. coli and C. jejuni co-colonization and the subsequent displacement of the established C. jejuni in broilers (El-Shibiny et al., 2007). It is thus possible that both the ceca and the litter environments may have had a role in the complex C. jejuni–C. coli interactions in the cecum (and thus litter).

The present study has also demonstrated that Campylobacter had a poor survival potential in litter. Unlike E. coli, the Campylobacter levels in litter were lower than its levels in ceca. Campylobacter is a fragile organism (Klancnik et al., 2009) and characterized by rapid die-off in litter (Chinivasagam, 2009). Campylobacter does not survive well outside the host and does not have the many key regulators of stress defense (such as oxidative and osmoprotection stationary phase
response) that are present in both *E. coli* and *Salmonella* (Park, 2002). The fact that it is a poor survivor outside and yet had the potential to reach these uniformly distributed high levels (log 8.0 to log 9.0 CFU/g in ceca) across the three litter treatments suggests that the microorganism’s emergence can be closely linked to a complex set of factors within the cecum, rather than the litter practices adopted.

In conclusion, these studies carried out under commercial farming conditions over a 2-year period have demonstrated that re-use of litter did not directly influence either the timing of emergence or levels of bacterial concentration achieved (*Campylobacter* and *E. coli*) or a predominance of a *Campylobacter* species detected when compared to the use of new bedding. The factors that influence the above are complex and not directly a feature of the litter re-use practices adopted.

**ACKNOWLEDGMENTS**

The financial support of the Rural Industries Research and Development Corporation, “Chicken Meat Research Program” is gratefully acknowledged. The guidance and support of Margaret MacKenzie (Director, Technical Services, Inghams Enterprises Pty. Ltd.) and the assistance of Kelly McTavish (Inghams Enterprises Pty. Ltd.) is gratefully acknowledged. The assistance provided and use of the farms of both Guy Douglas and Geoff O’Meara (Inghams farmers) is gratefully acknowledged.

**REFERENCES**

Allen, V. M., A. M. Ridley, J. A. Harris, D. G. Newell, and L. Powell. 2011. Influence of production system on the rate of onset of *Campylobacter* colonization in chicken flocks reared extensively in the United Kingdom. Br. Poult. Sci. 52:30–39.

Allen, V. M., H. Weaver, A. M. Ridley, J. A. Harris, M. Sharma, J. Emery, N. Sparks, M. Lewis, and S. Edge. 2008. Sources and spread of thermophilic *Campylobacter* spp. during partial depopulation of broiler chicken flocks. J. Food Prot. 71:264–270.

Allen, V. M., S. A. Bull, J. E. L. Corry, G. Domingue, F. Jorgensen, J. A. Frost, and T. J. Humphrey. 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int. J. Food Microbiol. 113:54–61.

Alter, T., and K. Scherer. 2006. Stress response of *Campylobacter* spp. and its role in food processing. J. Vet. Med. Series B-Infec. Dis. Vet. Public Health 53:351–357.

Apajalaiti, J. H. A., A. Kettunen, M. R. Bedford, and W. E. Holben. 2001. Percent G+C profiling accurately reveals diet-related succession in broiler chickens. Appl. Environ. Microbiol. 76:6572–6582.

Azcóituria, J. H., A. Kettunen, M. R. Bedford, and W. E. Holben. 2001. Percent G+C profiling accurately reveals diet-related succession in broiler chickens.

Barnes, E. 1972. The Avian intestinal flora with particular reference to the possible ecological significance of the cecal anaerobic bacteria. Am. J. Clin. Nutr. 25:1475–1479.

Best, E. L., E. J. Powell, C. Swift, K. A. Grant, and J. A. Frost. 2003. Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. FEMS Microbiol. Lett. 229:237–241.

Bull, S. A., V. M. Allen, G. Domingue, F. Jorgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, J. Gillard-King, and T. J. Humphrey. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. Appl. Environ. Microbiol. 72:645–652.

Chambers, J. R., and J. Gong. 2011. The intestinal microbiota and its modulation for *Salmonella* control in chickens. Food Res. Int. 44:3149–3159.

Chinivasagam, H. N. 2009. Re-use of chicken litter across broiler cycles – managing the food-borne pathogen risk. Final Report, Project No: 05–16, Poultry CRC, Australia. http://www.poultryhub.org/wp-content/uploads/2012/07/Final-Report-05-16.pdf

Chinivasagam, H. N., T. Tran, and P. J. Blackall. 2012. Impact of the Australian litter re-use practice on *Salmonella* in the broiler farming environment. Food Res. Int. 45:891–896.

Chinivasagam, H. N., T. Tran, L. Maddock, A. Gale, and P. J. Blackall. 2009. Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella*, *Campylobacter*, and *Escherichia coli*. Appl. Environ. Microbiol. 75:7417–7425.

Connell, S., K. G. Meade, B. Allan, A. T. Lloyd, E. Kenny, P. Cormican, D. W. Morris, D. G. Bradley, and C. O’Farrelly. 2012. Avian resistance to *Campylobacter jejuni* colonization is associated with an intestinal immunogen expression signature identified by mRNA sequencing. PLOS One 7:e40409.

Cox, N. A., L. J. Richardson, J. J. Maurer, M. E. Berrang, P. J. Fedorka-Cray, R. J. Buhr, J. A. Byrd, M. D. Lee, C. L. Hofacre, P. M. O’Kane, A. M. Lammerding, A. G. Clark, S. G. Thayer, and M. P. Doyle. 2012. Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J. Food Prot. 75:1896–1902.

Cressman, M. D., Z. T. Yu, M. C. Nelson, S. J. Moeller, M. S. Lilburn, and H. N. Zerby. 2010. Interrelations between the microorganisms in the litter and in the intestines of commercial broiler chickens. Appl. Environ. Microbiol. 76:6572–6582.

Daczkowska-Kozon, E. G., E. W. Sawicki, and K. Skotarczak. 2010. The ceca - niche supporting survival of *Campylobacter* spp. in commercially reared broiler chickens. Polish J. Food Nutr. Sci. 60: 265–271.

El-Shibiny, A., P. L. Connerton, and I. F. Connerton. 2005. Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens. Appl. Environ. Microbiol. 71:1259–1266.

El-Shibiny, A., P. L. Connerton, and I. F. Connerton. 2007. *Campylobacter* succession in broiler chickens. Vet. Microbiol. 125:323–332.

Fernandez, F., R. Sharma, M. Hinton, and M. R. Bedford. 2000. Diet influences the colonisation of *Campylobacter jejuni* and distribution of mucin carbohydrates in the chick intestinal tract. Cell Mol. Life Sci. 57:1793–1801.

GenStat. 2013. GenStat for Windows, Release 15.3. VSN International Ltd., Oxford.

Gong, J., R. J. Forster, H. Yu, J. R. Chambers, P. M. Sabour, R. Whincraft, and S. Chen. 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol. Lett. 208: 1–7.

Gornley, F. J., R. A. Bailey, K. A. Watson, J. McAdam, S. Avendano, W. A. Stanley, and A. N. M. Koerhuis. 2014. *Campylobacter* colonization and proliferation in the broiler chicken upon natural field challenge is not affected by the bird growth rate or breed. Appl. Environ. Microbiol. 80:6373–6378.

Hermans, D., F. Pasmans, W. Messens, A. Martel, F. Van Immerseel, G. Rasschaert, M. Heyndrickx, K. Van Deun, and F. Haezebrack. 2012. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. Vector-Borne Zoonotic Dis 12:89–98.

Ingmer, H. 2011. Challenges of *Campylobacter jejuni* in poultry production. Int. J. Food Microbiol. 145:S110.

Hetland, H., B. Svilhus, and A. Krogdal. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat Brit. Poult. Sci. 44:275–282.

Jaykus, K. 2003. Academic activities in food safety: centres, consortia, and initiatives, In Microbial Food Safety in Animal Agriculture. M. E. Torrence ed. Iowa State Press, Iowa, USA.

Klančnik, A., B. Guzej, P. Jammik, D. Vuckovic, M. Abram, and S. S. Mozina. 2009. Stress response and pathogenic potential of *Campylobacter jejuni* cells exposed to starvation. Res. Microbiol. 160:345–352.
CAMPYLOBACTER LEVELS IN LITTER AND CECA IN BROILERS

Line, J., K. Hiett, and A. Conlan. 2008. Comparison of challenge models for determining the colonization dose of Campylobacter jejuni in broiler chicks. Poult. Sci. 87:1700–1706.

LSU Ag Center. 2011. Making poultry litter safe for re-use. [makingpoultry+litter+safefor+reuse.htm accessed 25.11.14.]

Lu, J., U. Idris, B. Harmon, H. C., J. J. Maurer, and M. D. Lee. 2003a. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69:6816–6824.

Lu, J. R., S. Sanchez, C. Hofacre, J. J. Maurer, B. G. Harmon, and M. D. Lee. 2003b. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Appl. Environ. Microbiol. 69:901–908.

Montrose, M. S., S. M. Shane, and K. S. Harrington. 1985. Role of litter in the transmission of Campylobacter jejuni. Avian. Dis. 29:392–399.

Nather, G., T. Alter, A. Martin, and L. Ellerbroek. 2009. Analysis of risk factors for Campylobacter species infection in broiler flocks. Poult. Sci. 88:1299–1305.

Newell, D. G., K. T. Elvers, D. Dopfer, I. Hansson, P. Jones, S. James, J. Gittins, N. J. Stern, R. Davies, I. Connerton, D. Pearson, G. Salvat, and V. M. Allen. 2011. Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Appl. Environ. Microbiol. 77:8605–8614.

Park, S. F. 2002. The physiology of Campylobacter species and its relevance to their role as foodborne pathogens. Int. J. Food Microbiol. 74:177–188.

Payne, J. B., X. Li, F. B. O. Santos, and B. W. Sheldon. 2006. Characterization of Salmonella from three commercial North Carolina broiler farms. Int. J. Poult. Sci. 5:1102–1109.

Robinson, D. A. 1981. Infective dose of Campylobacter jejuni in milk. Brit. Med. J. 282:1584.

Rowell, J. G., and R. E. Walters. 1976. Analysing data with repeated observations on each experimental unit. J. Agric. Sci. 87:423–432.

Sahin, O., T. Y. Morishita, and Q. Zhang. 2002. Campylobacter colonization in poultry: sources of infection and modes of transmission. Anim. Health Res. Revs. / Conf. Res. Work. Anim. Dis. 3:95–105.

Shapiro, S. K., and W. B. Sarles. 1949. Microorganisms in the intestinal tract of normal chickens. J. Bact. 58:531–544.

Snelling, W. J., M. Matsuda, J. E. Moore, and J. S. G. Dooley. 2005. Under the microscope - Campylobacter jejuni. Lett. Appl. Microbiol. 41:297–302.

Svihus, B., H. Hetland, L. Mikkelsen, and S. Wu 2009. Role of voluntary litter consumption by broiler chickens on gut function and gut health. Final Report Project No: 06–18, Poultry CRC, Australia. http://www.poultryhub.org/wp-content/uploads/2012/07/06-18-Final-report.pdf

Torok, V. A., R. J. Hughes, K. Ophel-Keller, M. Ali, and R. MacAlpine. 2009. Influence of different litter materials onecal microbiota colonization in broiler chickens. Poult. Sci. 88:2474–2481.

Van Deun, K., F. Pasmans, R. Ducatelle, B. Flahou, K. Vissenberg, A. Martel, W. Van den Broeck, F. Van Immerseel, and F. Haesebrouck. 2008. Colonization strategy of Campylobacter jejuni results in persistent infection of the chicken gut. Vet. Microbiol. 130:285–297.

Wassenaar, T. M. 2011. Following an imaginary Campylobacter population from farm to fork and beyond: a bacterial perspective. Lett. Appl. Microbiol. 53:253–263.