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# Colonization of *Campylobacter* spp. in Broiler Chickens and Laying Hens Reared in Tropical Climates with Low-Biosecurity Housing

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**The onset and prevalence of *Campylobacter* colonization in broilers and layers at commercial farms with low biosecurity in tropical climates were tested. Despite the presence of positive animals at the same farms, the broiler flocks tested negative until, on average, 21 days. Prelaying flocks showed a higher prevalence than laying flocks.**

*Campylobacter jejuni* and *C. coli* are identified as major causes of food-borne disease in humans worldwide (1). Epidemiological studies have shown that handling and consumption of poultry meat are important risk factors for campylobacteriosis in humans (2).

Newly hatched chickens are free of *Campylobacter*. *Campylobacter* colonization of commercial broiler flocks could be detected after 1 to 2 weeks of age (3). As reasons for this lag phase in colonization, other than the likelihood of exposure to an infective dose of *Campylobacter*, the presence of maternal immunity (4) and a shift in the composition of the gut flora over time (5) have been suggested. In laying hens and broilers, both shedding and the fraction of colonized animals decline over time, with acquired immunity suggested as a reason (6, 7).

Studies in temperate countries have associated maternal immunity, acquired immunity, climatic conditions, poultry management systems, and the level of the biosecurity with the colonization and persistence of *Campylobacter* in chickens (5). Data on *Campylobacter* in poultry reared in tropical climates with open housing systems are scarce, and such data will add to the understanding of *Campylobacter* epidemiology. Hence, this study aimed to investigate the initial *Campylobacter* colonization of broilers and the prevalence of *Campylobacter* in laying hens under field conditions in commercial farm settings with low biosecurity in Sri Lanka, a tropical country in southern Asia.

As in many other tropical countries, in Sri Lanka, the deep-litter open-house system is commonly used in the poultry industry. Poultry, either broilers or laying hens, is reared in houses with half walls (approximately 0.5 m), with food and water provided inside the pen. Wire mesh is used to complete the walls and confine the birds in the pen to protect them from predators. However, contact with rodents, insects, wild birds, and other wild animals is unavoidable.

All farms, either broiler or layer, included in this study used the deep-litter open-house system, and the average number of animals per flock varied from 100 to 1,000. The management practices that were already in place were not changed to support this study. This included the use of commercially available feeds containing nonspecified coccidiostats and antibiotic growth promoters. The use of antibiotics otherwise was not monitored. *Campylobacter* colonization or shedding was tested by collecting cloacal swabs from a minimum of 10 birds selected randomly from each flock. Duplicate floor samples from each of five spots (four corners and the middle) in each pen were collected by using cotton swabs moistened with sterile saline before the chicks were introduced into the cleaned pens. Samples were transported at 10°C and processed within 24 h.

Cloacal swabs were cultured directly on modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid, Basingstoke, United Kingdom). Five floor swabs were directly streaked onto mCCDA agar. The remaining five were pooled and cultured in 10 ml of Preston enrichment broth (Oxoid) before plating on mCCDA agar. The enrichment procedure for floor samples and confirmation of the presence of *Campylobacter* were done according to the ISO standard (10272:2006). Colony morphology, motility, Gram staining, aerobic growth at 42°C, microaerobic growth at 25°C, catalase, and oxidase tests were used to identify the genus *Campylobacter*. Identification to the species level was performed by PCR (8). When all swabs collected at a given time point were culture negative, the flock or the pen was considered *Campylobacter* negative.

To detect the age of initial colonization of broilers, two farms were selected. One farm used the all-in, all-out system (farm A), while the other (farm B) used the multiple-age system. Both farms were mixed farms where broilers, layers, dairy cattle, and pigs were managed in separate but closely located (10 to 20 m) units. The animal caretakers took care of different animal species without changing their clothes or disinfecting their boots. Furthermore, the previous broiler flocks reared at those farms (less than a month ago) were found to be *Campylobacter* positive. During the study period of 1 year, farm A had four flocks with an empty period of 1 to 2 months between flocks. Farm B had 16 flocks partly overlapping in time (Fig. 1). All broilers were derived from eggs obtained from commercial parent flocks whose *Campylobacter* status was unknown. At both farms, the pens were cleaned thoroughly before a new flock was introduced and the chicks spent the first 2 weeks of

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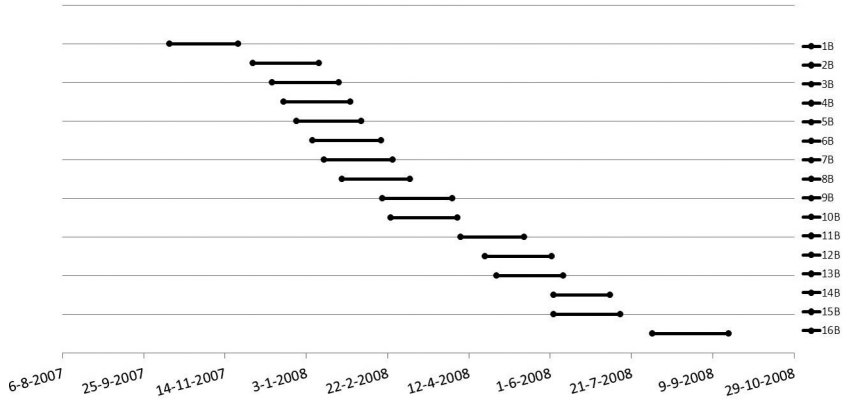


**FIG 1** The presence of different flocks at farm B versus time. The dates when the flocks were present are indicated on the *x* axis. Each bar represents an individual flock, which is indicated by a designation at the right.

their lives in a brooder. At farm A, the brooder was a small area of the pen, while at farm B, the brooder was a cage brooder situated in a separate location on the farm premises at a 10-m distance (not within the same pen).

*Campylobacter* was not detected in floor samples collected before chicks were introduced. Cloacal swabs collected every other day from the age of 1 day onward until the flock became positive for *Campylobacter* revealed that colonization of broilers was detected first on day 14 and then later. The mean ages at initial colonization at farms A and B were 23 and 20.3 days, respectively (Table 1). There was no statistically significant difference between farms A and B in terms of initial colonization (Mann-Whitney test at $P > 0.05$ using the R statistical software [http://www.R-project.org/]).

These data show a lag phase in colonization, which is in agreement with the published work based mainly on flocks kept in closed houses under well-maintained biosecurity conditions (3, 5). Of the isolates, 50% were *C. jejuni* and 50% were *C. coli*, which is comparable to data from a recent Europe-wide survey showing approximately 60% *C. jejuni* and 40% *C. coli* (9).

Exposure of a new flock to *Campylobacter* was most likely, in particular on farm B, with *Campylobacter*-positive birds present in adjacent pens at the time chicks were introduced into the poultry house from the cage brooder (Fig. 1). On farm A, there was no exposure from other broilers housed nearby (distance, >1 km) but pigs and cattle were present and tested positive for *Campylobacter* prior to this study. In general, other livestock has been identified as a risk factor for *Campylobacter* in broiler flocks (10). Furthermore, there was a huge number of flies on the premises that could act as vectors in the transmission of *Campylobacter* (11).

Biosecurity has been implicated as an important factor that influences the initial colonization of chicks, as it extends the lag phase in conventional broiler flocks (12). Two studies of free-range and organic management systems confirmed the presence of a lag phase in these systems (13, 14).

In the present study, the delay in colonization in the presence of exposure from positive birds and other animals strongly supports the hypothesis of a biological mechanism of protection in the first weeks. This study was designed not to identify this biological mechanism but as an observational study. No attempts were made to measure exposure quantitatively.

To study the relationship between the age of chickens and their *Campylobacter*-shedding status, a cross-sectional study was performed with 77 layer flocks from different farms at different production stages that were analyzed by using the same procedure described above. The overall prevalence of *Campylobacter* was 64%, with 90% at prelaying age (<140 days; $n = 20$), 60% in the first laying period (140 to 364 days; $n = 30$), and 52% in the

**TABLE 1** Age at initial colonization of broiler chicks naturally colonized with *Campylobacter* on farms A and B and dates of introduction of day-old chickens

| Location and flock or parameter | Date (day.mo.yr) of 1st day in cage brooder | Age (days) at: | | |
|---|---|---|---|---|
| | | Introduction into poultry house | Initial colonization | Slaughter date |
| Farm A[b] | | | | |
| 1A | NA[a] | 1 (15.10.07)[d] | 20 | 23.11.07 |
| 2A | NA | 1 (28.01.08) | 20 | 06.03.08 |
| 3A | NA | 1 (01.05.08) | 26 | 06.06.08 |
| 4A | NA | 1 (04.08.08) | 26 | 15.09.08 |
| Mean | | | 23 | |
| Farm B[c] | | | | |
| 1B | 11.10.07 | 15 | 20 | 22.11.07 |
| 2B | 01.12.07 | 15 | 20 | 11.01.08 |
| 3B | 13.12.07 | 15 | 20 | 23.01.08 |
| 4B | 20.12.07 | 16 | 20 | 30.01.08 |
| 5B | 28.12.07 | 14 | 14 | 06.02.08 |
| 6B | 07.01.08 | 15 | 22 | 18.02.08 |
| 7B | 14.01.08 | 15 | 22 | 25.02.08 |
| 8B | 25.01.08 | 15 | 19 | 07.03.08 |
| 9B | 19.02.08 | 16 | 26 | 02.04.08 |
| 10B | 24.02.08 | 16 | 22 | 05.04.08 |
| 11B | 07.04.08 | 15 | 22 | 16.05.08 |
| 12B | 22.04.08 | 15 | 17 | 02.06.08 |
| 13B | 29.04.08 | 15 | 19 | 09.06.08 |
| 14B | 03.06.08 | 15 | 23 | 08.07.08 |
| 15B | 03.06.08 | 15 | 23 | 14.07.08 |
| 16B | 03.08.08 | 15 | 19 | 19.09.08 |
| Mean | | | 20.3 | |

[a] NA, not applicable.
[b] All-in, all-out system.
[c] Multiple-age system.
[d] The date is in parentheses.

second laying period (>364 days; $n = 27$). A $\chi^2$ analysis showed that there were significant differences between the age groups ($\chi^2 = 7.62$, $P < 0.05$). Pairwise comparisons showed that, in particular, the prelaying age group differed significantly from the oldest group ($P < 0.05$). The difference between the prelaying and the first laying age groups bordered on significance ($P = 0.06$). Prevalence did not differ significantly between the two oldest groups. A cross-sectional survey conducted in Sweden involving 447 samples from laying hens has reported a similar finding (7). Our finding should be confirmed in a longitudinal study at the individual flock level.

In conclusion, this study shows that, under low biosecurity conditions in a *Campylobacter*-contaminated environment, broiler flocks remain negative until an average of 21 days, with a minimum of 14 days. At the layer flock level, *Campylobacter* prevalence is higher in prelaying flocks than in laying flocks. According to this study, the presence of a lag phase and development of resistance to *Campylobacter* colonization appear not to be restricted to commercial farming systems in the industrialized world.

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