Natural campylobacter colonization in chickens raised under different environmental conditions

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(Received 13 May 1985; accepted 14 November 1985)

SUMMARY

A cross-sectional study of 447 laying hens (age range 0–65 weeks) and a longitudinal study of 164 similar birds showed that Campylobacter jejuni was not present in the faeces of newly hatched chicks, but that colonization arose after 5–9 weeks. A survey of 250 broilers obtained from four breeders showed that all were negative for C. jejuni before and after slaughter at the age of 5 weeks. Once C. jejuni had appeared in a flock, it rapidly spread to virtually all birds, but at the age of 42 weeks only 20–46% of birds remained colonized, possibly as a result of having developed immunity. Birds housed in the protective environment of a laboratory still became colonized (after 9 weeks). The mode of infection is unknown, but water and food were bacteriologically negative and were deemed to be unlikely sources. Transmission via attendants, flies or other insects remain possibilities. It is concluded that prevention of colonization might be possible within the life-span of broiler chickens (5–7 weeks), but that it would be difficult to extend this period. There is a need to define how colonization arises so that the feasibility and cost of possible preventive measures can be assessed.

INTRODUCTION

As recently as a decade or so ago, Campylobacter jejuni was regarded as a bacterium whose importance was mainly confined to veterinary medicine. During recent years it has been shown that C. jejuni is a common cause of diarrhoea in humans throughout the world. In the western world it is the most common bacterial cause of diarrhoea and chickens are generally regarded as major sources of infection (Svedhem, Kajser & Sjögren, 1981; Skirrow, 1982; Blaser, Taylor & Feldman, 1983). It is a universal finding that most chicken carcasses sold for consumption are contaminated with C. jejuni. In order to decrease the risk of campylobacter in humans, the possibility of raising campylobacter-free chickens has been considered (Skirrow, 1982), but little is known of the epidemiology of the infection in chickens. Thus the aim of the present investigation was to study the natural colonization of chickens by C. jejuni during commercial breeding and raising.
Table 1. Study I: C. jejuni carriage rates in laying hens as shown by cross-sectional survey

<table>
<thead>
<tr>
<th>Age at sampling</th>
<th>Flock size</th>
<th>Breeding place</th>
<th>Breeding period</th>
<th>No. tested</th>
<th>No. positive for C. jejuni (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly hatched</td>
<td>8000</td>
<td>Hatchery</td>
<td>8 hours</td>
<td>106</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5 weeks</td>
<td>5000</td>
<td>Chicken farm I</td>
<td>0–5 weeks</td>
<td>142</td>
<td>7 (5)</td>
</tr>
<tr>
<td>16 weeks</td>
<td>5000</td>
<td>Chicken farm II</td>
<td>6–20 weeks</td>
<td>90</td>
<td>71 (72)</td>
</tr>
<tr>
<td>35 weeks</td>
<td>2000</td>
<td>Chicken farm II</td>
<td>20–35 weeks</td>
<td>50</td>
<td>16 (32)</td>
</tr>
<tr>
<td>65 weeks</td>
<td>2000</td>
<td>Chicken farm II</td>
<td>36–65 weeks</td>
<td>50</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Total number</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>447</td>
<td>105 (25)</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Three studies designed to look at different aspects of C. jejuni infection in chickens were performed.

Study I

This was a cross-sectional study of chickens of different ages bred for the production of laying hens. Rectal swabs were taken on a single day from 447 chickens whose ages ranged from 0 to 65 weeks. Depending on their age, they were kept at one of four locations: newly hatched chicks at a hatchery; 5-week-old birds at chicken farm I; 16-week-old birds in house A at chicken farm II; 35- and 65-week-old birds in laying accommodation in a separate building (house B) close to house A at chicken farm II (Table 1). At all four locations, flooring was of net to allow excrement to fall through.

Study II

This study was a longitudinal study of chickens housed under contrasting conditions. All 104 birds were from the same hatchery as in study I. Directly after hatching, 60 chicks were moved to the animal house at the Bacteriological Laboratory, University of Göteborg, and were kept there in one room throughout the study (Laboratory Group). The other 104 chickens were raised at the chicken farm, according to the ordinary routine there (Farm Group). Rectal swabs were taken at 2 to 4-week intervals from hatching to the age of 42 weeks.

The following differences in environmental conditions applied to the two groups. The Laboratory Group was kept in a room with an ordinary floor covered with wood shavings which was cleaned once a week. The Farm Group was kept in net cages (50 birds/cage) placed 1 m above the floor, which allowed the excrement to fall down through the net floor. When the birds were 20 weeks old they were all kept together in a big room, also with net floor. The food of the Laboratory Group contained 4% less raw protein than that of the Farm Group, and throughout the study contained a coccidiostatic substance (Amphriol®, Svenska Lantmannaföreningen, Sweden), which was added to the food of the Farm Group only until they were 5 weeks old. The food of the Farm Group was distributed automatically.
from big cisterns outside the buildings. Until the birds were 5 weeks the food line was placed just outside the cages. From 6 to 20 weeks the birds had their food from a line inside the cages. After 20 weeks the birds took their food and water from several containers hanging down from the ceiling in a big room. The water at the farm was taken from a pipeline directly connected to the mains water supply and distributed automatically into containers hanging down from each cage. The Laboratory Group had one common food and water container placed on the floor, which was refilled once a day. The water was drawn directly from the mains water supply for the community.

**Study III**

This was a survey of 250 broilers, obtained from four different breeders, raised for 35 days, and then slaughtered and prepared for sale. In each case 20000 broilers were kept in the same room, living directly on a floor covered with wood shavings. The room was not cleaned during the 35-day rising period. However, a very thorough cleaning of the room, including disinfection, was done between each cohort of birds. Water and food were distributed automatically as for the birds at the farm more than 20 weeks old, i.e. into containers hanging down from the ceiling.

Faecal specimens from 100 birds from one breeder were examined for the presence of *C. jejuni* on days 3, 10 and 35. Samples from these birds were also taken at the end of the slaughtering process.

One hundred and fifty chickens from the three other breeders (50 from each), were also investigated at the same slaughterhouse and the same slaughter occasion. Samples were taken from each bird just before slaughtering and at the end of the slaughtering process. A total of 700 samples were taken. All broilers were given the same food, which contained a coccidiostatic substance (Elancoban®, Svenska Lantmannaföreningen, Sweden) as well as an antimicrobial substance, virginiamycin (Stafac® Novo Industri, Malmö, Sweden). The main purpose of the virginiamycin was to promote growth (Cocito, 1979).

No special precautions were taken at any of the four breeder farms regarding attendants' clothes or boots for avoiding cross-contamination between groups of birds.

**Collection of samples from the birds**

At every examination one rectal swab was taken from each live chicken. The samples were taken with a sterile cotton-wool swab moistened in nutrient broth, transported in MSM-medium (Gästrin, Kallings & Marcetic, 1968) and cultivated within 4–6 h at the laboratory. Slaughtered birds were sampled with cotton-wool swabs rubbed over a 5 x 5 cm area both inside and outside of carcasses. These samples were transported and cultivated as described above.

**Collection of samples from the environment**

Environmental samples were taken in all studies from food, water, eggs, machines, cages, floors, walls, food and water containers in the rooms where the investigated birds were kept. Food and water samples were transported in sterile bottles containing brain–heart infusion broth and then incubated for 1 day before
being subcultured to selective agar plates. The other environmental samples were taken with moistened cotton-wool swabs rubbed over a 5 x 5 cm area and transported in MSM medium. The cultivation procedure and definition of campylobacter used were as outlined by Skirrow (1977).

RESULTS

In study I, no campylobacters were found in newly hatched chicks, but the frequency of C. jejuni isolations in 5-week-old chickens was 5%, rising to 72% at the age of 15 weeks, then decreasing to 32% at the age of 35 weeks and to 22% at 65 weeks (Table 1 and Fig. 1). C. jejuni was not found in the environmental specimens from the hatchery where birds were not colonized, but it was isolated from 10 out of 114 environmental samples from water or food basins in the cages of the colonized birds (Table 2).

In Study II, the birds in the Farm Group were colonized with C. jejuni at a frequency of 84% by the age of 7 weeks, reaching a 100% peak at 15 weeks of age. The chickens in the Laboratory group were first colonized at 9 weeks of age at a frequency of 20%, reaching a 94% peak at 11 weeks. Finally, at the age of 42 weeks, colonization had decreased to 20% for the Laboratory group and to 46% for the Farm Group (Fig. 1).

Of all 180 environmental samples taken from different localities in Study II, C. jejuni was found only in three cases (Table 2). These positive environmental samples originated from food and water basins in the cages of chicken Farm II, at a time when most of the birds were positive.

The environmental samples taken from the room where the Laboratory Group was kept were all negative. All tap-water samples analysed were negative as were all food samples taken from the package directly on arrival from the manufacturers.

In Study III, none of the 700 samples taken from 250 broilers (either live or when
Table 2. Occurrence of C. jejuni in environmental samples from different chicken-breeding locations and from slaughter-house

<table>
<thead>
<tr>
<th>Place of sampling</th>
<th>Study I: number of:</th>
<th>Study II: number of:</th>
<th>Study III: number of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Positive samples</td>
<td>Samples</td>
</tr>
<tr>
<td>Hatching farm</td>
<td>30</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>Chicken farm I</td>
<td>47</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>Chicken farm II</td>
<td>22</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Laying place</td>
<td>15</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Animal house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteriology laboratory</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Broiler farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughter-house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>10</td>
<td>180</td>
</tr>
</tbody>
</table>

DISCUSSION

There is abundant evidence that chickens commonly carry C. jejuni and that most commercially processed birds are contaminated (Svedhem, Kajser & Sjögren, 1981; Skirrow, 1982; Blaser, Taylor & Feldman, 1983). It would obviously be desirable to raise campylobacter-free chickens but little is known about the factors important in their colonization. In the present study we have shown that chickens begin life free of C. jejuni—a finding also reported by Neill, Campbell & Greene (1984) and Smitherman, Genigeorgis & Farver (1984). Once C. jejuni appeared, it spread rapidly throughout each flock to colonize virtually all birds, though after 42 weeks carriage rates had fallen to between 40% and 46% of birds, presumably owing to the development of immunity. None of the birds showed any sign of disease at the time of initial colonization, so C. jejuni presumably forms part of the normal intestinal flora of chickens. On the other hand, Neill, Campbell & Greene (1984) observed that the appearance of C. jejuni coincided with the appearance of wet litter in several of the chicken flocks they studied.

The source of infection is unknown. Keeping birds in the more controlled conditions of the bacteriology laboratory did not prevent infection, although it arose somewhat later (9 weeks) than in the farm birds (5 and 7 weeks). It is unlikely that the water, which was drawn directly from the mains supply, or food were sources of infection: both were bacteriologically negative for C. jejuni. Bacteria might have been brought in by the attendants on their hands, boots or clothing, since no special precautions were taken, but with the exception of six hens kept in the other end of the house the other animals kept in the laboratory were mice, rats, guinea-pigs and rabbits which are not commonly colonized with C. jejuni. No wild rodents or birds could get into the laboratory accommodation. Transmission via flies or ectoparasites such as fleas and mites is a possibility.

These results suggest that the number of C. jejuni organisms needed to initiate...
colonization is small and that their exclusion from large chicken-houses would require stringent conditions of containment. Yet the fact that colonization was absent in the 35-day-old broilers (Study III) and that it did not arise in the Study II farm birds until they were 7 weeks old is encouraging. Other possible reasons for absence of *C. jejuni* in the broilers are as follows. (1) The broilers were of a different genetic strain to the laying hens of Studies I and II and possibly less susceptible to infection; (2) The use of virginiamycin in the food given to the broilers but not the laying hens inhibited *C. jejuni*. (3) The use of wood shavings on the floor of the broiler house, which probably has a bactericidal effect on campylobacters as it does for salmonellae (Olesink, Snoeyenbos & Smyser, 1971); Snoeyenbos & Weinaek (1974) reported that campylobacters did not appear to spread so rapidly among chick reared on wood-shaving litter. (4) Methods used for isolation of campylobacters are not sensitive enough to detect small amounts of bacteria.

Others reported somewhat earlier colonization: Smitherman, Genigeorgis & Farver (1984) reported the absence of *C. jejuni* in five of six chicken-houses when birds were 40–46 days old, and Neill, Campbell & Greene (1984) reported colonization in most of their flocks within 4 weeks of hatching. Occasional flocks are reported that remain free from infection over many weeks.

Virtually all broilers in Sweden are slaughtered when they are 5 weeks old, so if infection can be prevented for this period, most of the chickens sold for consumption should be free of campylobacters, on the assumption that bacteria are not introduced during the slaughtering procedure.

We need to know how infection of commercially reared chicken flocks arises and then to assess the feasibility and cost of preventive measures. The results of our study suggest that prevention of infection in broilers, which are usually slaughtered at the age of 5 weeks, might be possible, but that it would be difficult to prevent infection in laying hens or older birds.

The investigation was supported by grants from the Swedish Medical Research Council (no. 16X–07169) and Swedish Council for Forestry and Agricultural Research (no. 598/84).

The skilful typing of Ms Anne-Bell Ek is very much appreciated.

REFERENCES


Campylobacter colonization in chickens

