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Natural campylobacter colonization in chickens raised under different environmental conditions

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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, Kaijser & 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*

Age at sampling	Flock size	Breeding place	Breeding period	No. tested	No. positive for *C. jejuni*(%)
Newly hatched	8000	Hatchery	8 hours	106	0 (0)
5 weeks	5000	Chicken farm I	0–5 weeks	142	7 (5)
16 weeks	5000	Chicken farm II house A	6–20 weeks	99	71 (72)
35 weeks	2000	Chicken farm II house B	20–35 weeks	50	16 (32)
65 weeks	2000	Chicken farm II house B	36–65 weeks	50	11 (22)
Total number	—	—	—	447	105 (25)

MATERIALS AND METHODS

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

Study 1

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 164 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 raising 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 5×5 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

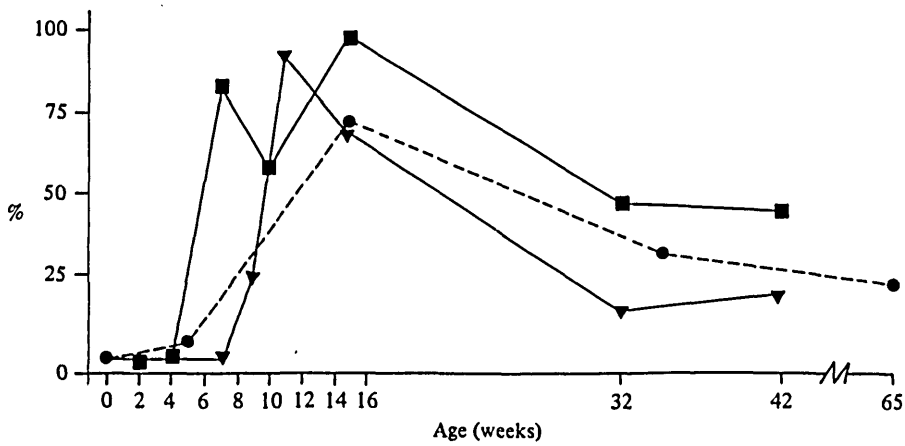

Fig. 1. Colonization frequency of campylobacter in the gut of chickens of different ages in study I and II. ○--●, Study I ($n = 447$); ■—■, Study II, Farm Group ($n = 104$); ▼—▼, Study II, laboratory group ($n = 60$).

being subcultured to selective agar plates. The other environmental samples were taken with moistened cotton-wool swabs rubbed over a 5 × 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*

Place of sampling	Study I: number of: Samples	Positive samples	Study II: number of: Samples	Positive samples	Study III: number of: Samples	Positive samples
Hatching farm	30	0	64	0	—	—
Chicken farm I	47	4	35	0	—	—
Chicken farm II	22	2	40	3	—	—
Laying place	15	4	7	0	—	—
Animal house						
Bacteriology laboratory	—	—	34	0	—	—
Broiler farm	—	—	—	—	47	0
Slaughter-house	—	—	—	—	41	0
Total	114	10	180	3	88	0

newly slaughtered), nor any environmental sample was positive for *C. jejuni* (Table 2).

DISCUSSION

There is abundant evidence that chickens commonly carry *C. jejuni* and that most commercially processed birds are contaminated (Svedhem, Kaijser & 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 & Weinack (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.

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