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Serotyping studies of campylobacter from naturally colonized chickens

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SUMMARY

Campylobacter jejuni/coli strains from 164 chickens were serotyped by the methods previously described by Penner *et al.* and Lior *et al.* The chickens were sampled during breeding from hatching to the age of 42 weeks. The birds were housed, in two separate groups, under different environmental conditions, (for comparison of the effect of hygienic precautions on the transmission of the bacteria during breeding). In the group where the hygienic conditions could be controlled to a greater extent, the chickens became colonized later in the breeding chain and with only one single campylobacter strain. Once campylobacter appeared in the group housed at the breeding farm, the birds were colonized with heterogenous antigenic strains. All birds in this group were colonized with more than one strain. By identifying campylobacter strains from chickens during breeding, it was shown that the hygienic conditions are very important for the production of chickens free from campylobacter, or for minimizing the number of colonizing strains.

INTRODUCTION

Previous studies have shown that chickens are among the most common sources of campylobacter diarrhoea in humans world-wide (Barot *et al.* 1983; Grant *et al.* 1980; Lindblom *et al.* 1986; Hood *et al.* 1988; Jones *et al.* 1984; Svedhem *et al.* 1981; Doyle, 1984). It has been shown that chickens are hatched *campylobacter* free, but are later easily and frequently colonized (Lindblom *et al.* 1986). Once present, the organisms reside in the intestinal tract of the chickens rather than in the tissues, and heavy surface contamination can occur on the surface during the slaughter process (Barot *et al.* 1983; Hood *et al.* 1988; Simmons & Gibbs, 1979).

The purpose of the present investigation was to study the conditions of and time schedule for campylobacter infection in newly hatched chickens with special reference to the occurrence of different serotypes, and to assess the significance of different hygienic procedures.

MATERIALS AND METHODS

Animals. One hundred and sixty-four chickens were hatched at a chicken hatchery (Lindblom *et al.* 1986). One hundred and four of the chickens were kept

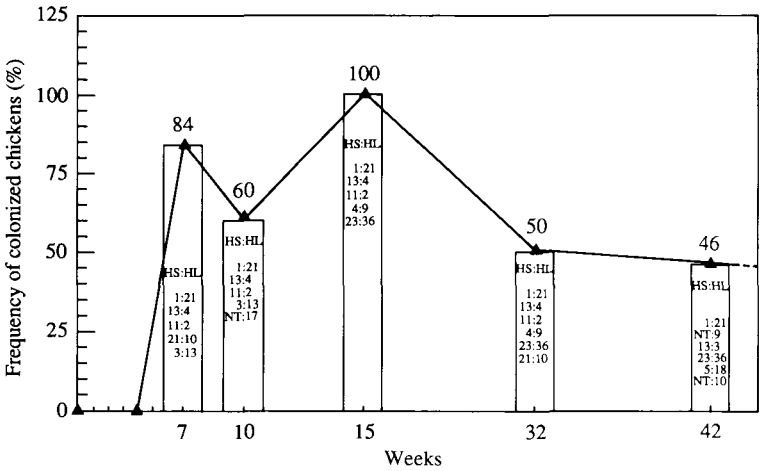

Fig. 1. Colonization frequency and serotype of campylobacter in the gut of chickens at different ages in the Farm group. ($N = 104$), $n = 28$. Bars indicate the range of different serotypes found at each sampling.

at an ordinary chicken breeding farm (Farm group), and 60 were brought up in our laboratory animal house, (Lab group). The distance between the two breeding places were approximately 100 km. The chickens in the two groups were given similar food except that of the Lab group contained 4% less raw protein than that of the Farm group and contained a coccidiostatic substance (Amphriol®, Svenska Lantmannaföreningen, Sweden), which was added to the food only until the birds were 5 weeks old. All other procedures were as identical as possible for the two groups.

Bacteria. Rectal swabs were taken from the chickens just after hatching, and at weeks 1, 4, 6, 7, 10, 11, 15, 32 and 42, and were cultured on Skirrow's campylobacter media. The definition of *Campylobacter jejuni/coli* was the one given by Skirrow (1977). Colonized chickens from the Farm group were sampled with rectal swabs at weeks 7, 10, 15, 32 and 42, and from the Lab group at weeks 9, 11, 15, 32 and 42. The campylobacter strains were stored by lyophilization of a streak with the needle from the whole primary, swarming culture or if necessary from a recultivation of the primary plate which was then handled in the same manner.

Serotyping. The serotyping of the HS antigens was performed by method of Penner & Hennessy (1980) and the HL antigens were identified by the method of Lior *et al.* (1982), using the antisera prepared by Kaijser & Sjögren, 1985. Twenty-eight strains were serotyped from the Farm group chickens and 21 strains from the Lab-group chickens.

RESULTS

The chickens in the Farm group were free from campylobacter for at least 4 weeks (Fig. 1). After 7 weeks, approximately 90% of the chickens were campylobacter positive. At this time five different campylobacter serotypes could be identified within the group. These chickens were colonized with antigenic

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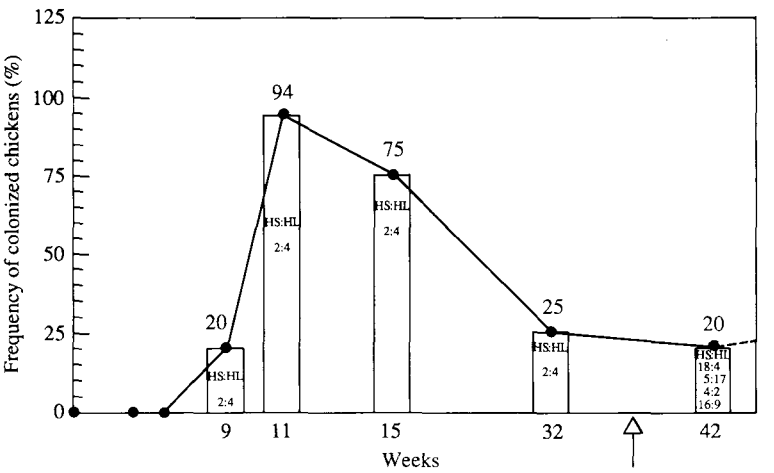

Fig. 2. Colonization frequency and serotype of campylobacter in the gut of chickens at different ages in the Lab group. (N = 60), $n = 21$. Bars indicate the range of different serotypes found at each sampling. Arrow indicates the time when the birds were transferred to the farm.

heterogenous strains of campylobacter during the whole study period. All birds in the group were colonized with more than one strain.

The Lab group was free from campylobacter for at least 6 weeks (Fig. 2). After 9 weeks, about 20% of the chickens were campylobacter positive and the figure increased to 94% after 11 weeks. In this group the chickens were colonized with only one campylobacter serotype (HS2:HL4). When the chickens were removed from the laboratory to an ordinary farm at the age of 42 weeks, the animals were immediately colonized with several other campylobacter serotypes.

In both groups the colonization frequency decreased with increasing age to a level of approximately 30–50%.

DISCUSSION

The process of colonization with campylobacters in chickens during breeding is not known in detail. It has been suggested that the bacteria are transferred from older animals in the area or transmitted via the farmers (Lindblom *et al.* 1986; Neill *et al.* 1984). It has also been suggested that rodents, flies, or wild animals might transmit campylobacter to newly hatched chickens (Walter *et al.* 1986; Smibert, 1978; Kanoyiannis *et al.* 1988). As a consequence of this, the hygienic procedures in the breeding farms seem important, in order to keep the frequency of campylobacter colonization as low as possible. The present study is in accordance with this hypothesis. In the farm, where the hygienic conditions could not be controlled in the same manner as in the laboratory animal house, the chickens were colonized earlier in the breeding chain and were colonized with several different campylobacter strains. In the laboratory animal house where as far as we know, no campylobacter bacteria existed, the chickens also became colonized though later in the breeding chain. These bacteria might have originated from the environs outside the animal house or from the other animals also kept in

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the laboratory and transferred by staff to the chickens. However, in the animal house only one campylobacter strain (HS2:HL4) colonized the animals. As soon as the animals were returned to a breeding farm, they were colonized with at least five different campylobacter strains.

By identifying both the HS and the HL antigens of the strains, the discriminate potential between different strains could be achieved, which is not the case when just one of the antigens is identified (Hood *et al.* 1988; Hutchinson *et al.* 1987). Combination testing also allows the recognition of the heterogeniety among the strains colonizing the chickens, especially in the Farm group (Fig. 1).

It is noteworthy that the most commonly found chicken strain HS2:HL4, is also a very frequent campylobacter serotype isolated both from outbreaks and sporadic cases in Sweden (Sjögren *et al.* 1988). This might suggest a direct link between human infections and local chickens as has been concluded from other epidemiologic investigations (Jones *et al.* 1984; Kanoyiannis *et al.*, 1988; Hood *et al.* 1988).

Since *Campylobacter jejuni/coli* is a very common cause of acute diarrhoea, and chickens are one of the most common reservoirs of this disease, it is of great benefit to the whole community to minimize the number of chickens being colonized during breeding (Thorén *et al.* 1988). Knowing that transmissible antibiotic plasmids could transfer resistant strains to humans, it is also important to limit the number of different strains colonizing the chickens during breeding (Tenover *et al.* 1985; Vanhoof *et al.* 1982; Taylor *et al.* 1981).

We conclude that hygienic conditions are very important for the breeding of chickens free from campylobacter. In agreement with earlier studies (Lindblom *et al.* 1986; Neill *et al.* 1984) we believe, that for the relatively short period of life of broiler chickens, around 35–40 days, it should be possible to breed the majority of the chickens campylobacter free and to minimize the number of colonizing strains.

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