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Epidemiology of *Campylobacter* spp. at two Dutch broiler farms

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SUMMARY

Broiler flocks on two Dutch poultry farms were screened weekly for the presence of campylobacter in fresh caecal droppings during eight consecutive production cycles. Hatchery and fresh litter samples were taken at the start of each new cycle. Water, feed, insects, and faeces of domestic animals, present on the farms were also included in the sampling. Penner serotyping of isolates was used to identify epidemiological factors that contribute to campylobacter colonization in the broiler flocks. Generally, broiler flocks became colonized with campylobacter at about 3–4 weeks of age with isolation percentages of 100%, and stayed colonized up to slaughter. A similar pattern of serotypes was found within the various broiler houses on one farm during one production cycle. New flocks generally showed also a new pattern of serotypes. Most serotypes isolated from the laying hens, pigs, sheep and cattle were different from those isolated from the broilers at the same time. Campylobacter serotypes from darkling beetles inside the broiler houses were identical to the ones isolated from the broilers. No campylobacter was isolated from any of the hatchery, water, feed or fresh litter samples. Conclusive evidence of transmission routes was not found, but results certainly point towards horizontal transmission from the environment. Horizontal transmission from one broiler flock to the next one via a persistent contamination within the broiler house, as well as vertical transmission from breeder flocks via the hatchery to progeny, did not seem to be very likely.

INTRODUCTION

Live poultry is often found to be colonized by campylobacter. The birds are healthy carriers of this bacterium, which may be found at counts of 10^6–10^9 colony forming units per gram of faeces [1, 2]. Campylobacter colonization of live poultry may affect public health in two ways: (1) direct effect of the organism causing disease in workers at farms or processing plants [3, 4], and (2) contamination of consumer-ready poultry products, which in turn may cause food-borne illness [5–7].

Intervention strategies have to be developed in order to reduce contamination

rates and thus the risk of campylobacter infections in humans. Possible means of preventing campylobacter colonization of live broiler flocks have to be found in the first place. Unfortunately, the epidemiology of campylobacter colonization in poultry is not yet fully understood. Feed, water, domestic animals, insects, rodents and wild birds have all been suggested as possible sources of horizontal transmission [2, 8, 9]. Vertical transmission from campylobacter-positive breeder flocks via the egg to their progeny, has not been found to be very likely [10–12].

A longitudinal study on the presence of campylobacter in broiler flocks and environmental sources was carried out at two Dutch poultry farms. Campylobacter isolates were serotyped according to Penner [13, 14] in order to identify epidemiological factors contributing to the campylobacter colonization of broiler flocks on these farms.

MATERIALS AND METHODS

Farm descriptions

Presence of campylobacter on two broiler farms (Farms D and E) was monitored from November 1989 until January 1991, during eight consecutive broiler production cycles. A cycle is the rearing period of broilers of approximately 6 weeks in a separate chicken house on one farm, starting at the entry into the house and finishing at slaughter of all birds.

Both farms used fresh straw for litter on concrete floors. After each cycle all used litter was removed and the houses, including the drinkers and feeding system, were cleaned and disinfected. The broiler houses remained empty for at least 1 week before new flocks arrived. Separate boots and clothing, as well as a boot disinfection bath, were present in each house on both farms. However there was some doubt about the proper use of these facilities. Both farms operated a system in which a proportion of the flock was removed from the houses at about 5 weeks of age, with the remaining birds being slaughtered at about 6 weeks.

Four broiler houses were present on Farm D. House D1 and D2 were completely separate buildings, but houses D3 and D4 were connected by a joint feeding room. All broiler flocks were of the same breed and were obtained from a single hatchery. Flock sizes ranged from 20000 birds in houses D3 and D4 to 30000 birds in houses D1 and D2. Chicks received a combined spray vaccination against Newcastle Disease and Infectious Bronchitis at the hatchery, and a vaccination via the drinking water against Gumboro disease at 12 days of age. The birds were fed pelletized feed: a starter feed for the first 17 days after hatching, a grower feed for the next 3 weeks, and a finisher feed during the last 5–7 days before slaughter. Furazolidone was added to the starter feed at a concentration of 200 mg/kg for the first 7 days of life to prevent *Escherichia coli* infections. The coccidiostats nicarbazin (125 mg/kg) and salinomycin (60 mg/kg) were added to the starter feed and the grower feed, respectively. Avoparcine was added to all types of feed as a growth promoter at a concentration of 10 mg/kg. Chicks received tap water; with bell-type drinkers being used in houses D1, D3 and D4, and a nipple system in house D2. Rodents (mainly mice) and insects (flies and darkling beetles) were controlled with appropriate chemicals. On a separate location 0·5 km away, this farmer managed a flock of 9000 laying hens.

Three separate broiler houses (houses E1 to E3) were present on Farm E. Broilers were always of the same breed and were obtained from a single hatchery. A total of 30000 chicks was placed in house E1 and after about 2 weeks, half the number of birds was moved to house E2. Broiler flocks in house E3 consisted of about 30000 birds. Vaccination was carried out as described for Farm D, except that the combined NCD/IB vaccination took place at the farm. The birds were fed pelletized feed, but no detailed information on the types of feed was available. Chicks received tap water, with bell-type drinkers being used in houses E1 and E2, and a nipple system in house E3. In addition to the broilers, pigs, cattle (milking cows) and sheep were present on this farm. Rodents and insects were controlled by mechanical means (however, these were unsuccessful at least for darkling beetles and lesser mealworms in houses 1 and 3.

Sampling

Broiler flocks on Farms D and E were screened for the presence of *Campylobacter* spp. during eight consecutive cycles. Hatchery debris and fluff, and paper pads from the transport coops for the day-old chicks, as well as fresh litter were sampled at the start of each new production cycle.

Ten to 30 samples of fresh caecal droppings were taken weekly from each of the broiler houses. Samples were collected with sterile swabs. When campylobacter was not detected on the farm, 30 caeca from the broiler flock were taken at slaughter for examination.

Environmental samples examined for the presence of campylobacter included tap water, feed, insects and faeces of domestic animals, present on the farms. Water and feed samples were taken during the first or second week of each block cycle and supplementary water samples were taken on Farm E during the fifth week of each cycle in the different houses. Water and feed samples were taken from the separate storage bins, before coming in contact with the broilers. Darkling beetles and lesser mealworms (*Alphitobius diaperinus* and larvae) were collected every time they were observed on the floor or walls of a broiler house. Laying hens were examined by taking individual swab samples of fresh caecal droppings. Pigs, sheep and cattle were sampled by taking pools of fresh faecal material. Examination of samples for the presence of *Campylobacter* spp. was carried out within 2 h after sample collection.

Isolation and serotyping of Campylobacter *spp.*

Swab samples were directly streaked on campylobacter blood-free selective medium (CCD-agar, Oxoid CM 739), with cefoperazone (Oxoid SR 125, 32 mg/l medium) and actidione (Sigma, 100 mg/l medium) as selective agents. CCD-agar plates were incubated micro-aerobically in anaerobic jars with CampyPak Plus (BBL 71045). Incubation was at 37 °C for 2 days. Suspect colonies were examined under the microscope for the typical corkscrew shape and rapid, darting motility. A latex agglutination test (Meritec Campy, Meridian Diagnostics) was used for final confirmation.

Hatchery, fresh litter, water and feed samples were diluted 1:9 (w/v) in the selective enrichment medium CCD-broth (per litre: 25 g nutrient broth no. 2 [Oxoid CM 67], 4 g bacteriological charcoal [Oxoid L9], 3 g casein hydrolysate

[Oxoid L41], 1 g sodium deoxycholate [Merck 6504], 0·25 g ferrous sulphate [Merck 3965], 0·25 g sodium pyruvate [Merck 6619] and the selective agents cefoperazone and actidione as described for the CCD-agar). Water and feed were tested in portions of 25 ml and 25 g, respectively. The external surface of the collected insects was disinfected with 96% alcohol, and one sample of 10–50 insects was ground and transferred into 10 ml of CCDB. One gram of faecal samples of pigs, sheep and cattle was brought into 10 ml of CCDB. The CCD-broth was incubated microaerobically for 2 days at 42 °C. A loopful of broth was then subcultured on a CCD-agar plate and handled as described for the swab samples.

Over 800 campylobacter isolates from both broilers and environmental sources were serotyped by using the heat-stable serotyping system according to Penner [13]. This system was modified to include absorption of antisera and the use of a pooled typing system as described by Jacobs-Reitsma and colleagues [14].

RESULTS

Screening of broiler farms for presence of Campylobacter *spp.*

Figures 1 and 2 summarize the results of campylobacter isolations from the broilers during eight consecutive cycles on Farms D and E, respectively. As shown in Figure 1, broiler flocks in houses D3 and D4 arrived on the farm one week before the flocks in houses D1 and D2. Broiler flocks in house E3 arrived 3–5 days after the flocks in houses E1 and E2 (Fig. 2).

Campylobacter was isolated from 18 of the 32 flocks examined (56%) on Farm D. On Farm E, 20 of the 22 flocks studied (91%) were found to be colonized by campylobacter. The earliest detection of campylobacter was in cycle 4 on Farm E, when the broilers were 13 days old. Only one out of the 30 samples was campylobacter-positive at that time, but the isolation rate was 100% (30/30) 7 days later.

Generally, the broilers became colonized by campylobacter at about 3–4 weeks of age. Once campylobacter had entered a flock, all broilers became colonized within 1 week and remained colonized up to slaughter with isolation rates close to 100%.

The results of campylobacter isolations from environmental samples are summarized in Table 1. No campylobacter was isolated from any of the hatchery or paper pad samples. Campylobacter was not isolated from any of the fresh litter, water and feed samples examined in this study.

The laying hens on Farm D were found to be colonized by campylobacter throughout the whole survey. The same was true for the pigs on Farm E. However, campylobacter was isolated less frequently from the sheep on this farm, and only isolated twice from the milking cows (both times during cycle 8). Campylobacter isolations from the domestic animals were independent of the presence of campylobacter in the broiler flocks.

No darkling beetles or lesser mealworms were observed in house E2, but in houses E1 and E3 they were found during all cycles. Especially during summer (cycles 4–6) their presence was abundant, but generally they could not be captured before the second or third week of a production cycle, as they were still hiding in

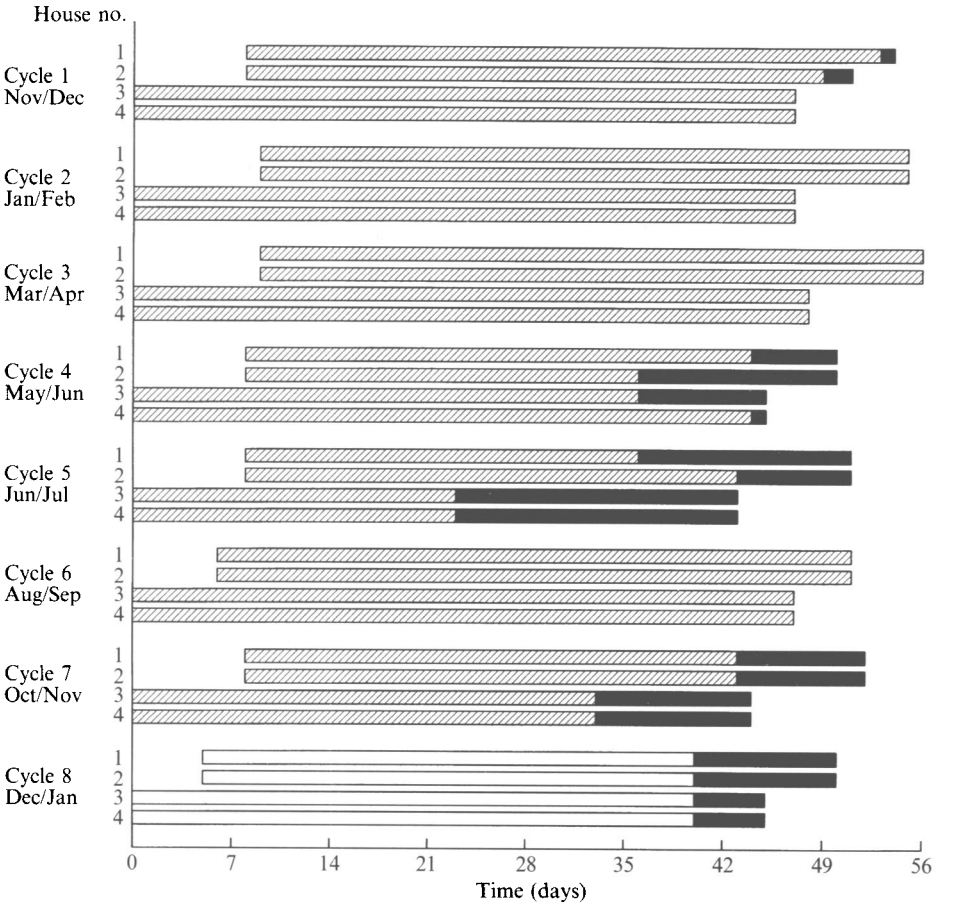

Fig. 1. Results of campylobacter screening of broiler flocks in Farm D. houses D1–D4 (November 1989 to January 1991): (▨), −; (■), +; (□), not determined.

the roofs. *Campylobacter* species were isolated from these insects on several occasions, but never before the broilers in that particular house were found to be colonized by campylobacter.

Serotyping of campylobacter isolates

A total of 809 campylobacter isolates from both broilers and environmental samples were serotyped; 14% of these isolates were not typable with the 65 sera available.

On Farm D, 15 different serotypes were isolated from the various broiler flocks, with a maximum of four different serotypes within one flock at one sampling time. Generally, a similar pattern of serotypes was found in the four separate broiler houses within one production cycle. Consecutive cycles, however, generally showed a different pattern of serotypes. A total of 13 different serotypes was isolated from the laying hens on the farm, and up to nine different types were detected at one sampling time. Some serotypes (e.g. O1,44; O24; O60) were found at different sampling times, whereas other types (e.g. O30, O2; O65; O51) were detected only once during the whole survey. Some serotypes were isolated from

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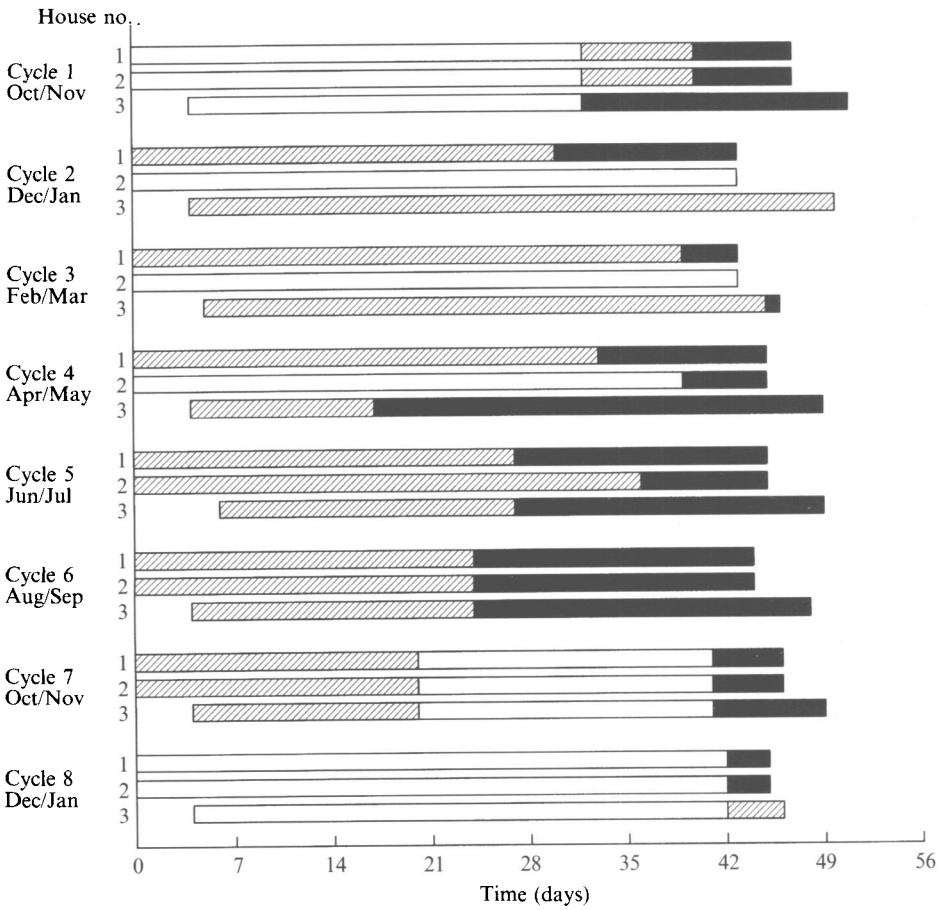

Fig. 2. Results of campylobacter screening of broiler flocks in Farm E, houses E1–E3 (October 1989 to January 1991): (▨). − : (■). + : (□). not determined.

Table 1. *Isolation of campylobacter from environmental samples of Farms D and E*

Type of sample	Campylobacter isolations
Hatchery samples (total)	0/187*
Paper pads (total)	0/115
Fresh litter (total)	0/48
Water (total)	0/80
Feed (total)	0/56
Laying hens (Farm D)	71/82
Pigs (Farm E)	40/45
Sheep (Farm E)	10/17
Cattle (Farm E)	12/26
Insects (Farm E, houses 1 and 3)	36/104

* Number of campylobacter positive samples/number of samples tested.

both the broilers and the laying hens on this farm, but not always at the same sampling time. Some serotypes frequently isolated from the broilers (like O49; O37; O56) were not isolated at all from the laying hens.

Fifteen different serotypes were isolated from the various broiler flocks on Farm

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E, and up to five different serotypes could be isolated from one flock at the same time. Serotype O5g was the most frequently isolated serotype from the broiler flocks on Farm E and was found during cycles 3, 4, 6, 7 and 8. Apart from the frequent isolation of this particular serotype, the pattern of serotypes found during the various cycles was quite variable. The serotype pattern in all three broiler houses within one production cycle was much more similar, although even within one cycle a change in serotype pattern was sometimes observed.

Serotypes isolated from the darkling beetles and lesser mealworms were also present in the broilers during the same cycle and mostly in the house from where the insects were collected.

Ten different serotypes were observed in the 32 pig isolates that were serotyped. Only three of them were also found in the broilers, but O5g (cycle 4) and O30 (cycle 6) were isolated at the same sampling time from both pigs and broilers. Due to the low isolation rate from sheep and cattle on Farm E, only a small number of isolates from these animals were serotyped. Only O5g was isolated from both cattle and broilers at the same sampling time (cycle 8).

DISCUSSION

Cambylobacter species were frequently isolated from the broiler flocks but never before the birds were 2 weeks of age. Isolation of campylobacters generally occurred between 3 and 4 weeks of age. Once campylobacter was isolated from a flock, all broilers in that particular broiler house became colonized within 1 week, and isolation rates in the flocks remained at 100% up to the time of slaughter. Similar findings, both on the time of first detection and isolation rates within flocks, have been reported in other longitudinal studies [1, 15, 16]. However, not all broiler flocks were found to be campylobacter-positive at the end of the production cycle. Moreover, both campylobacter-positive and -negative flocks could be present at the same time on one farm, even up to slaughter. Therefore, campylobacter-free rearing of broiler flocks can be achieved and is certainly an important tool in the prevention of human campylobacteriosis via poultry meat products.

No campylobacter species were detected in any of the hatchery samples. Broilers from identical parent flocks were found to be colonized in one production cycle and campylobacter-free in another. These data do not support the likelihood of vertical transmission as an important pathway; although Dutch breeder flocks are frequently found to be campylobacter carriers [17]. Other studies report similar findings with respect to vertical transmission [10–12]. Since campylobacter was not isolated from any of the fresh litter, water and feed samples, these factors did not seem to be of major importance for transmission of campylobacter in this study.

The majority of campylobacter-positive broiler flocks were colonized with more than one serotype at the same time, as has been observed in other studies [15, 18]. The serotype distribution within a flock sometimes changed during the production cycle. Most likely, this reflects the constant flow of other campylobacters entering a broiler house, which may result in a dominance of a newly introduced serotype. Laboratory experiments with broilers and challenge with two different campylobacter serotype strains showed a complete dominance of one within

1 week. Results on repeated serotyping of several campylobacter strains did not suggest any serotype instability within the strains as a possible cause [19].

Similar campylobacter serotype patterns were found at the same time in different flocks (from different breeder flocks) in one farm. This clearly indicates transmission from the same environmental sources or cross-contamination from adjacent houses. Consecutive cycles generally showed a different serotype pattern, so carry over of campylobacter within a house from the previous flock did not seem to occur on these farms.

The laying hens on Farm D were found to be permanent carriers of a number of campylobacter serotypes and should therefore be regarded as a potential source of campylobacter contamination for broiler flocks on this farm. The laying hens might occasionally have been the source of the campylobacter contaminations (cycle 5, serotype O5g or cycle 7, serotype O14). Most other serotypes, frequently found in the broilers, were not isolated from the layers at any time. Other, undefined, sources are therefore suspected to play an important role in transmission of campylobacter.

In accordance with other studies [12, 20], pigs (Farm E) were found to be permanent carriers of a variety of campylobacter serotypes. However, a similar serotype in both pigs and broilers during one cycle was detected only twice (cycle 4: O5g and cycle 6: O30). No clear correlation, except for the O5g isolates in cycle 8, was found between serotypes from sheep and cattle and serotypes from broilers on Farm E. But this might partly be due to the relatively low number of sheep and cattle isolates that could be tested. Nevertheless, these ruminants, as well as pigs and laying hens, cannot be excluded as potential sources of campylobacter infection for broilers on a farm.

In contrast to the findings of Jones and colleagues [21], campylobacter was isolated on several occasions from the internal contents of darkling beetles and lesser mealworms, although this never occurred before the organism was also isolated from the broilers. Identical serotypes were isolated from both the insects and broilers within broiler houses E1 and E3. This might indicate an infection route from insects to broilers, but the reverse infection route from broilers to insects is just as likely. More detailed studies are needed to determine the survival and colonization potential of campylobacter in these insects under the less optimal conditions of an empty (and generally cold) broiler house.

The results from this study, and in particular the large number of different campylobacter serotypes isolated from both broiler flocks and environmental sources, indicate the complexity of campylobacter epidemiology in broiler flocks. Conclusive evidence for certain transmission routes was not found, but the results point towards horizontal transmission from the environment. Neither horizontal transmission from one broiler flock to the next due to a persistent contamination within the broiler house, nor vertical transmission from breeder flocks via the hatchery to their progeny, seem to be very likely.

Intervention procedures against horizontal transmission have to be studied further and the effectiveness of strict hygienic practices during the whole production period, such as was described as being successful in small scale experiments by Van de Giessen and colleagues [12] and Humphrey and co-workers [22], should be evaluated on a larger scale.

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