*Epidemiol. Infect.* (1998), **121**, 57–66. Printed in the United Kingdom 

# Reduction of campylobacter infections in broiler flocks by application of hygiene measures

A. W. VAN DE GIESSEN[1]*, J. J. H. C. TILBURG[1], W. S. RITMEESTER[1] AND J. VAN DER PLAS[2]

[1]*Microbiological Laboratory for Health Protection, National Institute of Public Health and the Environment, 3720 BA Bilthoven, the Netherlands*
[2]*Department of Bioprocessing and Biomonitoring, TNO Nutrition and Food Research, 3700 AJ Zeist, the Netherlands*

(*Accepted 10 February 1998*)

## SUMMARY

Transmission routes of *Campylobacter* spp. in broilers and possibilities for prevention of infections were studied on two Dutch broiler farms. The occurrence of *Campylobacter* spp. was studied in successive broiler flocks, in the environment of the farms and in some of the parent flocks involved. Isolates of *Campylobacter* spp. were typed by using randomly amplified polymorphic DNA (RAPD) analysis. The results indicate that broiler flocks become infected from environmental sources. The typing results suggest that on one farm transmission of *Campylobacter* spp. occurred from cattle to broilers via the farmer's footwear. After several campylobacter positive broiler cycles hygiene measures, including thorough cleaning and disinfection procedures, change of footwear at the entrance of each broiler house, control of vermin and other hygienic precautions, were introduced on both farms in order to prevent transmission of *Campylobacter* spp. from the farm environment to the broilers. The results indicate that the application of hygiene measures significantly reduced campylobacter infections of broiler flocks on both farms.

## INTRODUCTION

Within the past two decades *Campylobacter jejuni/coli* has risen from anonymity as a veterinary pathogen to recognition as a major cause of human diarrhoeal illness in many industrialized countries [1]. In the Netherlands, for example, a population study revealed that the annual incidence of campylobacter enteritis approximates 200 cases per 100000 persons (2%), some 300000 cases per year [2]. While most outbreaks of campylobacter infection have been associated with consumption of raw milk and untreated water, sporadic cases constituting the vast bulk of the campylobacter infections have predominantly been associated with consumption of poultry meat [1, 3–7]. In recent studies conducted in the Netherlands *Campylobacter* spp. were isolated from 82% of the broiler flocks examined at slaughter [8], and from 40% of chicken products in retail stores [9]. Although considerable efforts have been made to improve slaughterhouse hygiene, contamination of broiler carcasses from the intestinal contents is not likely to be prevented [10, 11]. Therefore, a reduction in human campylobacteriosis should be pursued by reducing campylobacter infection in broiler flocks. Several studies have been performed to elucidate the transmission routes of *Campylobacter* spp. in broiler flocks. Results unanimously indicate that vertical transmission of the organism from parent flocks to progeny

* Author for correspondence.

via the eggs is not likely to occur and that flocks become infected from environmental sources [12–18]. Potential environmental sources include the poultry house environment, the old litter, untreated drinking water, other farm animals, domestic pets, rodents, insects wild birds, farm workers, equipment and transport vehicles [19–21]. Different approaches for control of campylobacter infection in poultry flocks are being considered. Administration of defined competitive exclusion floras as well as administration of dietary carbohydrates were shown to be effective in reducing *Campylobacter jejuni* colonization in poultry flocks [22–24]. Also, it was demonstrated that antibody treatment can effectively increase the dose required to colonize chicks with *C. jejuni* [25]. Further, attempts have been made to control the organism by improving the on-farm hygiene. In a British study [26] it was demonstrated that dipping boots in phenolic disinfectant before workers entered broiler houses either delayed or prevented colonization. In the present study, the effect of hygiene measures applied on broiler farms to prevent transmission of *Campylobacter* spp. from the farm environment to the broilers was tested.

## MATERIALS AND METHODS

### Farms

The present study, which was conducted during 1992 and 1993, involved two broiler farms, farm C and farm D, belonging to the same poultry company. On farm C and D three and two broiler houses (C1, C2 and C3, and D1 and D2)- respectively were present. On both farms the all-in all-out system was used, which meant that the broiler houses were depopulated, left empty for 2 weeks and restocked simultaneously. In the first week after depopulation the broiler houses were cleaned and disinfected as a matter of routine. The broilers were raised for a period of 6 weeks until slaughter. However, at an age of 5 weeks a part of the flocks (approximately 25%) was already slaughtered. On both farms wood shavings were used for litter and the broilers were supplied with tap water and pelletized feed. Rodents, mainly mice, and insects, mainly flies and darkling beetles, were controlled by using appropriate chemicals. Apart from poultry, on farm C about 100 pigs were kept in a pig house adjacent to the broiler houses. Further, a dog and pigeons were present on this farm. On farm D cattle, including dairy cows and calves, were held adjacent to the broiler houses. Also, a pony, a dog and some cats were present on this farm.

### Sampling

During nine successive broiler cycles (except for cycle 8 on farm D) the broiler flocks present in broiler houses C1, C2, C3, D1 and D2 were sampled. Flock sizes were 10000–25000 birds. The broiler flocks were derived from various breeder flocks. The flocks were sampled a few days before depopulation. From each flock 10 pooled samples of faeces were collected at random by dipping a sterile cotton swab into 10 fresh caecal droppings. The swabs were transferred into tubes containing 2 ml of phosphate buffered saline and transported to the laboratory for examination on *Campylobacter* spp. The number of samples taken per flock (100) enabled the detection of flocks with 3% infected birds, at a confidence level of 95%. Also, swabs of fresh caecal droppings were taken from the parent flocks of the broilers in houses C1, C2 and C3 in broiler cycles 6 and 7. Further, on both farms diverse samples were taken from the environment of the broiler houses. In general, these samples were taken shortly before or shortly after delivery of new flocks. On farm C these environmental samples included swabs of fresh faecal material from the pigs, the dog and the pigeons, large gauze swabs from the floors and walls of the broiler houses taken a few days after cleaning and disinfection, samples of beetles and drinking water collected from the nipple system in each broiler house. At chick delivery large gauze swabs were taken from the delivery boxes and from the paper pads in the boxes as well as from the delivery lorry. At the time of partial depopulation such swabs were taken from the lorry and from the slaughterhouse crates. On farm D the environmental samples included swabs of fresh faecal material from the cattle, the pony and the dog, large gauze swabs from the floors and wall of the broiler houses taken a few days after cleaning and disinfection, samples of feed, insects (flies and beetles), drinking water collected from the nipple system in each broiler house, a sample from the caecal contents of a mouse and large gauze swabs from the farmer's boots worn by the farmer on the farm yard at the time of sampling.

### Isolation of Campylobacter *spp.*

Examination of samples for the presence of *Campylobacter* spp. was carried out within 2 h of collection.

https://doi.org/10.1017/S0950268898008899 Published online by Cambridge University Press

Swab samples were plated out directly by swabbing the surface of campylobacter blood-free selective agar (Oxoid CM 739 with cefoperazone [32 mg/l] and cycloheximide [100 mg/l]). Large gauze swabs and portions of 1 g of feed were added to 20 ml of campylobacter selective enrichment broth (THAL) (thioglycolate broth [BBL 11260] with 5% lysed horse blood, vancomycin [0·04 g/l], polymyxin-B-sulphate [0·01 g/l], trimethoprim [0·02 g/l], cycloheximide [0·1 g/l], cephalothin [0·1 g/l], and lauryl sulphate [1 g/l]) [27]. Volumes of 1 l of drinking water were filtrated through a 0·2 $\mu$m Millipore filter after which the filters were transferred into 20 ml of THAL. Samples of insects were ground and transferred into 20 ml of THAL as well. After incubation at 37 °C for 24 h in a microaerobic atmosphere (7% $O_2$, 10% $CO_2$ and 83% $N_2$) the broth were streaked onto campylobacter blood-free selective agar. Plates were incubated microaerobically at 42 °C for 48 h. From each plate one colony suspected of being a *Campylobacter* spp. was transferred onto campylobacter blood-free agar (Oxoid CM 739 without antibiotics), which was incubated under similar conditions. Characteristic colonies were examined under a phase-contrast microscope for typical spiral-shaped cells and rapid motility. One presumed *Campylobacter* spp. isolate per positive sample was DNA-typed as described below. The identity of a proportion of these isolates was confirmed by using a polymerase chain reaction (PCR) technique as described below.

## RAPD analysis

Isolates of *Campylobacter* spp. from the broiler flocks and the environment of the broiler farms were typed by using randomly amplified polymorphic DNA (RAPD) analysis as described by Mazurier and colleagues [28]. In summary, colonies of *Campylobacter* spp. were inoculated in Brain Heart Infusion (BHI)-broth and incubated microaerobically at 37 °C for 24 h. One ml of the culture was centrifuged, cells were washed in saline and centrifuged again and the cell pellet was resuspended in 500 $\mu$l of distilled water. Then the suspension was heated to 100 °C for 3 min and centrifuged. The supernatant was adjusted to an $O.D._{260}$ of 0·15 by dilution in distilled water and 5 $\mu$l was used in the amplification reaction. In this reaction one primer, HLWL85, was used. After amplification the reaction mixture was electrophoresed on a 1·6% agarose gel, stained with ethidium bromide and photographed under UV-transillumination to visualize the RAPD pattern.

Table 1. *DNA sequences of the primers used in the* C. jejuni–C. coli *multiprimer PCR assay*

| Code | 5′→3′ nucleotide sequence |
|---|---|
| COL1 | AGGCAAGGGAGCCTTTAATC |
| COL2 | TATCCCTATCTACAAATTCGC |
| JUN3 | CATCTTCCCTAGTCAAGCCT |
| JUN4 | AAGATATGGCACTAGCAAGAC |

## PCR identification

In consideration of the high discriminatory power of RAPD analysis, it was assumed that *Campylobacter* spp. of an identical RAPD-type simultaneously isolated from the same source belong to the same species. Therefore, from each set of presumed *Campylobacter* spp. of the same RAPD-type simultaneously isolated from the same source, a single or a few isolates were selected for identification to species. The selected isolates were identified by using a multiprimer PCR technique for identification of *C. jejuni* and *C. coli* [29, 30]. In this assay, specific PCR amplification of *C. jejuni* and *C. coli* was performed with primers based on the nucleotide sequences of monospecific probes, selected for specificity from *C. jejuni* and *C. coli* DNA fragment libraries [31]. The DNA sequences of the primers are shown in Table 1. These primersets, JUN3 + JUN4 and COL1 + COL2 respectively, were combined into a *C. jejuni* – *C. coli* multiprimer PCR assay. The reaction mixtures (25 $\mu$l) had final concentrations of 20 mM Tris/HCl pH 8·3, 50 mM KCl, 3 mM $MgCl_2$, 0·01% gelatin and 0·1 mM of each deoxyribo-nucleotide triphosphate. The reaction mixtures each contained 1·0 unit of *Thermus aquaticus* (Taq) DNA polymerase (Perkin–Elmer), 50 pmol of each primer and 5 $\mu$l of DNA-extracts prepared as described for the RAPD analysis. All reactions were performed in a Perkin–Elmer DNA thermal cycler model 480, using a touch-down protocol [32]. The PCR protocol included: a first step of 5 min at 94 °C; 2 cycles of consecutively 1 min at 94 °C, 1 min at 64 °C, and 1 min at 72 °C; 2 cycles of consecutively 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C; 2 cycles of consecutively 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C; 2 cycles of consecutively 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C; 2 cycles of consecutively 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C; 30 cycles of

https://doi.org/10.1017/S0950268898008899 Published online by Cambridge University Press

Table 2. *Occurrence of* Campylobacter *spp. in successive broiler flocks on farm C before* (*cycles 1–6*) *and after* (*cycles 7–9*) *introduction of control measures*

| | Broiler house C1 | | Broiler house C2 | | Broiler house C3 | |
|---|---|---|---|---|---|---|
| Broiler cycle no. | RAPD type (no. of samples) | *Cam.* species (no. of isolates) | RAPD type (no. of samples) | *Cam.* species (no. of isolates | RAPD type (no. of samples) | *Cam.* species (no. of isolates) |
| 1 | ND* | | ND (10) | | ND (10) | |
| 2 | A (10) | *C. jejuni* (2) | A (10) | *C. jejuni* (2) | A (5) | *C. jejuni* (2) |
| | | | | | B (2) | *C. coli* (2) |
| | | | | | ND (3) | |
| 3 | ND (10) | | C (2) | *C. jejuni* (2) | C (2) | *C. jejuni* (2) |
| | | | D (2) | *C. coli* (1) | F (1) | *C. jejuni* (1) |
| | | | E (2) | *C. coli* (1) | G (3) | *C. jejuni* (3) |
| | | | ND (4) | | ND (4) | |
| 4 | ND (10) | | A (9) | *C. jejuni* (2) | A (8) | *C. jejuni* (1) |
| | | | ND (1) | | ND (2) | |
| 5 | ND (10) | | H (2) | *C. jejuni* (1) | J (2) | *C. jejuni* (1) |
| | | | I (2) | *C. jejuni* (1) | K (2) | *C. jejuni* (1) |
| | | | ND (6) | | ND (6) | |
| 6 | H (2) | *C. jejuni* (1) | H (4) | *C. jejuni* (1) | H (4) | *C. jejuni* (1) |
| | I (2) | *C. jejuni* (1) | I (2) | *C. jejuni* (1) | L (2) | *C. coli* (1) |
| | L (2) | *C. coli* (1) | L (2) | *C. coli* (1) | ND (4) | |
| | ND (4) | | ND (2) | | | |
| 7 | ND (10) | | ND (10) | | ND (10) | |
| 8 | ND (10) | | ND (10) | | ND (10) | |
| 9 | CNT† (10) | NI‡ | CNT (10) | NI | ND (10) | |

* ND, *Campylobacter* spp. were not detected.
† CNT, *Campylobacter* spp. were detected but were not RAPD-typed.
‡ NI, *Campylobacter* sp. was not identified.

consecutively 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C; and a final step of 10 min at 72 °C.

PCR products were separated on 1·6% agarose gels and stained with ethidium bromide. The species classification was deduced from the size of the amplification product. The PCR product for *C. jejuni* is 773 bp in length, that for *C. coli* 363 bp. The specificity of this *C. jejuni*/*coli* assay was shown to be the same as that of the *C. jejuni* and *C. coli* probes.

### Application of control measures

After broiler cycle 6 on farm C and broiler cycle 2 on farm D control measures were introduced to prevent campylobacter infection of the broiler flocks. These measures included thorough cleaning and disinfection of the broiler houses including the entrance room between successive broiler cycles. For disinfection 20% formalin and halamid were used on farm C and D respectively. In order to improve the effect of cleaning and disinfection, cracks in the floors and walls of the broiler houses were repaired as good as possible. Further, a hygiene barrier was constructed in the entrance room of each broiler house. At the hygiene barrier footwear had to be changed for separate boots and separate overalls had to be used by every person entering the broiler house. These boots were cleaned and disinfected regularly. The farmer was encouraged to wash his hands before and after tending the broiler flocks. Children and pets were not permitted in the broiler houses. Separate tools had to be used per broiler house and introduction of possibly contaminated materials, such as the slaughterhouse crates used at partial depopulation, was not permitted. The control of rodents and insects was emphasized and the broiler houses were made vermin-proof as much as possible. The farm yard was kept tidy and was cleaned and disinfected regularly.

## RESULTS

### Farm C

The results of the examination on *Campylobacter* spp. of the broiler flocks in broiler houses C1, C2 and C3 are presented in Table 2. In the first broiler cycle *Campylobacter* spp. were detected in none of the

https://doi.org/10.1017/S0950268898008899 Published online by Cambridge University Press

Table 3. *Occurrence of* Campylobacter *spp. in the environment of farm C and in parent flocks of the broilers on farm C*

| Material or animal | Broiler cycle no.* | RAPD type (no. of samples) | *Cam.* species (no. of isolates) | Material or animal | Broiler cycle no. | RAPD type (no. of samples) | *Cam.* species (no. of isolates) |
|---|---|---|---|---|---|---|---|
| Pigs | 3 | M (1) | *C. coli* (1) | Parent flocks | 6 | AA (4) | *C. jejuni* (1) |
| | | N (1) | *C. coli* (1) | of broilers | | AB (1) | *C. jejuni* (1) |
| | | O (1) | *C. coli* (1) | in house C1 and C2 | | AC (1) | *C. jejuni* (1) |
| | 5 | P (1) | *C. coli* (1) | | | AD (1) | *C. coli* (1) |
| | | Q (1) | *C. coli* (1) | | | AE (1) | *C. jejuni* (1) |
| | 7 | Q (1) | *C. coli* (1) | | | AF (5) | *C. coli* (1) |
| | | R (1) | *C. coli* (1) | | | AG (1) | NI‡ |
| | | S (3) | *C. coli* (3) | | | AH (1) | *C. jejuni* (1) |
| | 8 | Q (4) | *C. coli* (4) | | | ND (5) | |
| | | S (6) | *C. coli* (6) | | 7 | CNT§ (45) | *C. jejuni* (1) |
| | | T (1) | *C. coli* (1) | | | ND (5) | |
| | | U (3) | *C. coli* (3) | of broilers | 6 | ND (20) | |
| | | V (2) | *C. coli* (2) | in house C3 | 7 | CNT (26) | *C. jejuni* (1) |
| | | W (1) | *C. coli* (1) | | | ND (4) | |
| | | X (1) | *C. coli* (1) | | | | |
| | | Y (2) | *C. coli* (2) | Drinking water | 7 | ND (3) | |
| | | Z (2) | *C. coli* (2) | | | | |
| Broiler houses | 4 | ND† (3) | | Paper pads | 7 | ND (15) | |
| floors and walls | 5 | ND (3) | | | 8 | ND (4) | |
| | 8 | ND (3) | | Delivery boxes | 8 | ND (3) | |
| Dog | 5 | ND (1) | | Delivery lorry | 8 | ND (2) | |
| Pigeons | 5 | ND (1) | | Lorry used at partial | 8 | CNT (1) | *C. coli* (1) |
| | 7 | ND (2) | | depopulation | | ND (1) | |
| Beetles | 7 | ND (2) | | Slaughterhouse crates | 8 | CNT (5) | *C. coli* (1) |
| | | | | used at partial depopulation | | ND (3) | |

* Environmental samples were taken shortly before or shortly after delivery of new flocks.
† ND, *Campylobacter* spp. were not detected.
‡ NI, *Campylobacter* species was not identified.
§ CNT, *Campylobacter* spp. were detected but were not RAPD-typed.

Table 4. *Occurrence of* Campylobacter *spp. in successive broiler flocks on farm D before* (*cycles 1–2*) *and after* (*cycles 3–9*) *introduction of control measures*

| | Broiler house D1 | | Broiler house D2 | |
|---|---|---|---|---|
| Broiler cycle no. | RAPD type (no. of samples) | *Cam.* species (no. of isolates) | RAPD type (no. of samples) | *Cam.* species (no. of isolates) |
| 1 | BA (3) | *C. jejuni* (1) | BB (10) | *C. jejuni* (1) |
| | BB (7) | *C. jejuni* (1) | | |
| 2 | BA (1) | *C. jejuni* (1) | BA (1) | *C. jejuni* (1) |
| | BB (9) | *C. jejuni* (2) | BB (9) | *C. jejuni* (2) |
| 3 | BB (10) | *C. jejuni* (1) | BA (9) | *C. jejuni* (1) |
| | | | BB (1) | *C. jejuni* (1) |
| 4 | ND* (10) | | BA′§ (10) | *C. jejuni* (1) |
| 5 | ND (10) | | BA (1) | *C. jejuni* (1) |
| 6 | BB′† (10) | *C. jejuni* (1) | ND (10) | |
| 7 | ND (10) | | ND (10) | |
| 8 | NS‡ | | NS | |
| 9 | ND (10) | | ND (10) | |

* ND, *Campylobacter* spp. were not detected.
† RAPD-pattern BB′ is only slightly different from pattern BB.
‡ NS, not sampled.
§ RAPD-pattern BA′ is only slightly different from pattern BA.

Table 5. *Occurrence of* Campylobacter *spp. in the environment of farm D*

| Material or animal | Broiler cycle no.* | RAPD type (no. of samples) | *Cam.* species (no. of isolates) |
|---|---|---|---|
| Cattle | 2† | BB (4) | *C. jejuni* (3) |
| | | ND‡ (6) | |
| | 4 | ND (5) | |
| | 6 | BB (1) | C. jejuni (1) |
| | | ND (12) | |
| | 7 | CNT§ (1) | *C. jejuni* (1) |
| | | ND (4) | |
| Pony | 4 | ND (1) | |
| Dog | 4 | ND (3) | |
| Insects | 5† | BA (1) | *C. jejuni* (1) |
| | | ND (1) | |
| | 6 | ND (3) | |
| Broiler houses floors and walls | 6 | ND (20) | |
| Feed | 6 | ND (2) | |
| Drinking water | 6 | ND (2) | |
| Mouse | 6 | ND (1) | |
| Farmer's boots | 6‖ | BB′¶ (1) | *C. jejuni* (1) |
| | | ND (3) | |
| | 7 | ND (1) | |

* In general environmental samples were taken shortly before or shortly after delivery of new flocks.
† Samples were taken at the end of the broiler cycle.
‡ ND, *Campylobacter* spp, were not detected.
§ CNT, *Campylobacter* spp. were detected but were not RAPD-typed.
‖ Samples were taken during the second week of the broiler cycle.
¶ RAPD-pattern BB′ is only slightly different from pattern BB.

https://doi.org/10.1017/S0950268898008899 Published online by Cambridge University Press

broiler flocks present. During broiler cycles 2–6 *Campylobacter* spp. were isolated from all successive broiler flocks in houses C2 and C3 and from flocks 2 and 6 in house C1. After introduction of control measures on farm C (after cycle 6) *Campylobacter* spp. were not detected in any of the broiler flocks during cycles 7 and 8. However, in broiler cycle 9 the organism was isolated again from the flocks present in houses C1 and C2.

RAPD-analysis of *Campylobacter* spp. isolates from the broiler flocks in cycles 2–6 yielded 12 different RAPD-types (A to L). Isolates of eight RAPD-types were speciated as *C. jejuni*, whereas isolates of four types (B, D, E, L) appeared to be *C. coli*. Five RAPD-types (A, C, H, I, L) were found in two or three of the flocks present in the same broiler cycle. Further, RAPD-type A appeared in both broiler cycle 2 and 4, while types H and I were found both in cycle 5 and 6. The results of the samples taken from the environment of farm C and from parent flocks are presented in Table 3. *Campylobacter* spp. were consistently isolated from the pigs present on this farm. RAPD-analysis of pig isolates yielded 14 types (M–Z) which were different from the types found in the broiler flocks. Isolates of these types were all speciated as *C. coli*. Also, *Campylobacter* spp. were isolated from the parent flocks of the broilers in houses C1 and C2 in cycles 6 and 7 and from the parent flocks of the broilers in house C3 in cycle 7. RAPD-analysis of isolates from the parent flocks of the broilers in houses C1 and C2 in cycle 6 yielded eight types which were different from the types found in their progeny. Further, *Campylobacter* spp. were isolated from the slaughterhouse crates as well as from the lorry used at partial depopulation of the broiler houses in cycle 8 (isolates not RAPD typed). *Campylobacter* spp. were not detected in samples taken from the floors and walls of the empty broiler houses, the dog, pigeons, beetles, drinking water, chick delivery boxes, paper pads and the delivery lorry.

## Farm D

The results of the broiler flocks in broiler houses D1 and D2 are presented in Table 4. *Campylobacter* spp. were isolated from all flocks during the first two broiler cycles. After introduction of control measures on farm D (after cycle 2) *Campylobacter* spp. could not be detected in flocks 4, 5, 7 and 9 in house D1 and in flocks 6, 7 and 9 in house D2 (flocks in cycle 8 were not sampled). Thus, the organism was isolated from flocks 3 and 6 in house D1 and from flocks 3–5 in house D2.

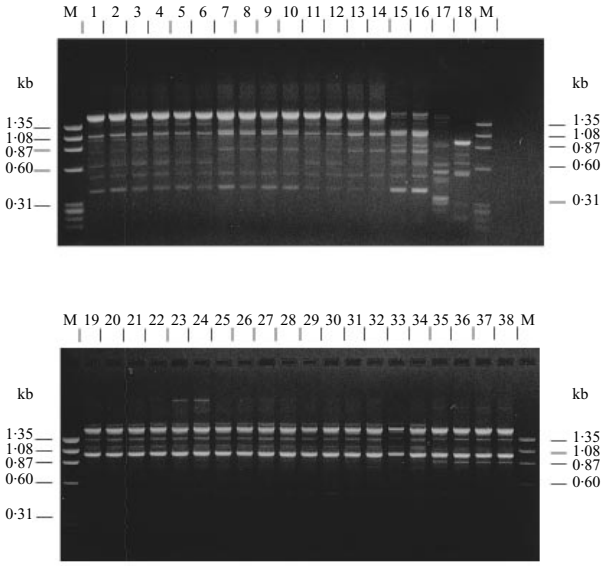


**Fig. 1.** RAPD patterns obtained with primer HLWL85 for *Campylobacter* spp. isolates from broiler flocks and the environment of farm D (two successive lanes contain duplicate samples). Lanes 1–8 and 11–14, type BA from broilers; lanes 9–10, type BA from insects; lanes 15–16, type BA′ from broilers; lanes 17–18, controls; lanes 19–26 and 29–32, type BB from broilers; lanes 27–28 and 33–34, type BB from cattle; lanes 35–36, type BB′ from farmer's boots; lanes 37–38, type BB′ from broilers. Lanes m contain a molecular weight marker (*Hae*III-digested fX174 DNA).

RAPD-analysis of the *Campylobacter* spp. isolates from the broiler flocks yielded two clearly distinctive RAPD-patterns, BA and BB, as well as two patterns, BA′ and BB′, that were only slightly different from types BA and BB, respectively (Fig. 1). Types BA and BB were found in several of the successive flocks in broiler houses D1 and D2. The results of the samples taken from the environment of farm D are presented in Table 5. *Campylobacter* spp. were isolated form the cattle during broiler cycles 2, 6 and 7. RAPD-analysis of cattle isolates yielded type BB. Further, *Campylobacter* spp. of type BA were isolated from a sample of insects taken in the broiler houses at the end of broiler cycle 5. Finally, *Campylobacter* spp. of type BB′ were obtained from the farmer's boots worn on the farm yard at the time of sampling during the second week of broiler cycle 6. The RAPD-patterns concerned are presented in Figure 1. Isolates of types BA, BA′, BB and BB′ were all speciated as *C. jejuni*. *Campylobacter* spp. were not isolated from samples taken form the floors and walls of the empty broiler houses, feed, drinking water, the pony, the dog and the mouse.

https://doi.org/10.1017/S0950268898008899 Published online by Cambridge University Press

## DISCUSSION

In the present study hygiene measures were introduced on two farms that harboured campylobacter positive broiler flocks for the preceding five and two broiler cycles respectively. The measures were directed to prevent transmission of *Campylobacter* spp. from the farm environment, including the broiler houses itself, to the broilers. After introduction of the control measures the percentage of campylobacter positive flocks decreased from 66% (12/18) to 22% (2/9) at farm C and from 100% (4/4) to 42% (5/12) at farm D. Due to the preventive measures it was achieved on both farms to raise flocks free of *Campylobacter* spp. for at least two successive broiler cycles. From these results we conclude that the application of hygiene measures significantly reduced campylobacter infections in broiler flocks on both farms. However, the reappearance of positive flocks after introduction of the control measures indicates that this intervention strategy may reduce but can not totally exclude the risk of campylobacter infections in broiler flocks. It must be realized that strict maintenance of the hygiene measures on the farm level is difficult. The results of this study are in agreement with the results of our recent epidemiological study in which application of specific hygiene measures including the use of separate boots in the broiler house, footbath disinfection and handwashing before tending the broilers was associated with a reduced risk of campylobacter infections in broilers [33]. Moreover, these results confirm the results obtained by Humphrey [26] as well as the preliminary findings of a previous study [16] suggesting that campylobacter infections in broilers can be reduced by application of hygiene measures.

As far as the infection routes are concerned the conclusion drawn above indicates that the broiler flocks became infected by horizontal pathways from the farm environment. The presence of *Campylobacter* spp. of an identical RAPD-type (BB) in the cattle and in several of the successive broiler flocks on farm D clearly suggests that the cattle were a major source of infection to the broilers on this farm. This result is in agreement with our recent epidemiological study in which the presence of other farm animals on the broiler farm, including pigs, cattle, sheep and fowl others than broilers, was strongly associated with an increased risk of campylobacter infection in broilers [33]. The finding of a closely related RAPD-type (BB′) in one of the flocks may be an indication for genetic instability of *Campylobacter* spp., which phenomenon has recently been discussed by Goossens and colleagues [34]. The isolation of this RAPD-type (BB′) from both the farmer's boots and a broiler flock during the same broiler cycle suggests that on farm D transmission of *Campylobacter* spp. from the cattle to the broilers (and vice versa) occurred via the farmer's footwear. The isolation of an identical RAPD-type (BA) from insects and broilers on farm D suggests that insects may have served as vehicle of transmission as well on this farm, for example from one broiler flock to the next in the same broiler house. On farm C, transmission from the pigs to the broilers would not seem unlikely. However, the results show a lack of correlation between the types found in the broilers (in majority *C. jejuni*) and the types isolated from the pigs (all *C. coli*). The discrepancy could be explained by the high diversity of *Campylobacter* spp. genotypes that are found to be present in pigs [35] and preference of *C. jejuni* and *C. coli* to grow out in poultry and pigs respectively. If transmission of *Campylobacter* spp. from the pigs to the broilers on farm C did occur, the *C. coli* types predominating in the pigs may not have grown out in the broilers, whereas other *C. coli* types and possibly *C. jejuni* types which may have been present in the pigs below the detection level may have emerged in the broilers. On the other hand, other sources, such as vermin, may have been involved in the infection of the broilers on farm C. Since rodents could not be trapped easily and only a single mouse (from farm D) was examined in the present study, a possible role of these animals in the transmission of *Campylobacter* spp. to broilers cannot be excluded. Further, the isolation of *Campylobacter* spp. from a lorry and slaughterhouse crates used at partial depopulation suggests that such materials, if not properly cleaned and disinfected, may easily transmit *Campylobacter* spp. to broilers during transport as well as to broiler farms. Since these crates usually are brought into the broiler houses at partial depopulation, the broilers remaining in the houses until the end of the broiler cycle may become infected. The difference in RAPD-types between *Campylobacter* spp. isolates from parent flocks and isolates from their progeny and the finding of campylobacter negative broiler flocks originating from campylobacter positive parent flocks provide a further indication that vertical transmission of infection does not occur. The fact that *Campylobacter* spp. could not be detected in the empty broiler houses after cleaning and disinfection suggests that transmission via the broiler houses did not occur. Finally, the feed, whose dryness adversely

affects survival of *Campylobacter* spp., and the drinking water (mains) would seem unlikely sources of infection.

*Campylobacter jejuni/coli* is recognized as a common human enteropathogen in many developed countries and poultry meat plays a dominant role in the transmission of the organism to humans [1]. Thus, an intervention strategy preventing colonization of broiler flocks with *Campylobacter* spp. may substantially improve this public health situation. The results of the present study indicate that broiler flocks become infected from environmental animal sources and suggest that campylobacter infections in broiler flocks can significantly be reduced by application of hygiene measures. However, since application of hygiene measures can reduce but not exclude campylobacter infection in broilers, a combination of these measures with other preventive measures reducing *Campylobacter* spp. in broilers, such as administration of a competitive exclusion flora [22–24] or dietary carbohydrates [23], should be considered. Control of *Campylobacter* spp. on the farm combined with monitoring of broiler flocks at the end of the broiler period and a slaughtering procedure in which campylobacter negative flocks are daily slaughtered before campylobacter positive flocks (logistic slaughtering) in order to avoid cross-contamination should form an effective intervention strategy to reduce campylobacter contamination of poultry meat.

## ACKNOWLEDGEMENTS

This study was carried out on behalf of the Dutch Veterinary Public Health Inspectorate (VHI). We wish to thank Coppens Feed Ltd. for their enthusiastic cooperation in the study. Also, thanks are due to M. Hout (VHI) and T. Leenen (Regional Veterinary Public Health Inspectorate 's-Hertogenbosch) for sampling. Further, we acknowledge Prof Dr J. H. J. Huis in 't Veld for his constructive criticism.

## REFERENCES

1. Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, Tompkins LS, eds. *Campylobacter jejuni* current status and future trends. Washington DC: American Society for Microbiology, 1992.
2. Wit MAS de, Hoogenboom-Verdegaal AMM, Goosen ESM, Sprenger MJW, Borgdorff MW. Een bevolkingsonderzoek in vier regio's in Nederland naar de incidentie en ziektelast van gastro-enteritis en van *Campylobacter* –en *Salmonella*-infectie. Bilthoven: National Institute of Public Health and Environmental Protection, 1996; RIVM report no. 149101014.
3. Oosterom J, den Uyl CH, Bänffer, JRJ, Huisman J. Epidemiological investigations on *Campylobacter jejuni* in households with a primary infection. J Hyg 1984; **92**: 325–32.
4. Hopkins RS, Olmsted R, Istre GR. Endemic *Campylobacter jejuni* infection in Colorado: identified risk factors. Am J Publ Health 1984; **74**: 249–50.
5. Deming MS, Tauxe RV, Blake PA, *et al*. *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. Am J Epidemiol 1987; **126**: 1220.
6. Brieseman MA. A further study on the epidemiology of *Campylobacter jejuni* infections. New Zealand Med J 1990; **103**: 207–9.
7. Kapperud G, Skjerve E, Bean NH, Ostroff SM, Lassen J. Risk factors for sporadic *Campylobacter* infections: results of a case control study in southeastern Norway. J. Clin Microbiol 1992; **30**: 3117–21.
8. Jacobs-Reitsma WF, Bolder NM, Mulder RWAW. Caecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: a one-year study. Poultry Science 1994; **73**: 1260–6.
9. Zee H van der, de Boer E, Jansen JT. *Salmonella* spp., *Salmonella enteritidis* and *Campylobacter* in poultry products in The Netherlands in 1991–1993. De Ware(n) Chemicus 1994; **24**: 81–5.
10. Oosterom J, Notermans S, Karman H, Engels GB. Origin and prevalence of *Campylobacter jejuni* in poultry processing. J Food Protect 1983; **46**: 339–44.
11. Genigeorgis C, Hassuney M, Collins P. *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughtering. J Food Protect 1986; **49**: 895–903.
12. Doyle MP. Association of *Campylobacter jejuni* with laying hens and eggs. Appl Environ Microbiol 1984; **47**: 533–6.
13. Shanker S, Lee A, Sorrell TC. *Campylobacter jejuni* in broilers: the role of vertical transmission. J Hyg 1986; **96**: 153–9.
14. Annan-Prah A, Janc M. The mode of spread of *Campylobacter jejuni/coli* to broiler flocks. J Vet Med B 1988; **35**: 11–8.
15. Kazwala RR, Collins JD, Hannan J, Crinion RAP, O'Mahony H. Factors responsible for the introduction and spread of *Campylobacter jejuni* infection in commercial poultry production. Vet Rec 1990; **126**: 305–6.
16. Giessen A van de, Mazurier S-I, Jacobs-Reitsma W, *et al*. Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. Appl Environ Microbiol 1992; **58**: 1913–7.
17. Pearson AD, Greenwood M, Healing TD. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. Appl Environ Microbiol 1993; **59**: 987–96.

18. Jacobs-Reitsma WF, Giessen AW van de, Bolder NM, Mulder RWAW. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol Infect 1995; **114**: 413–21.
19. Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev 1983; **5**: 157–75.
20. Kapperud G, Skjerve E, Vik L, *et al*. Epidemiological investigation of risk factors for *Campylobacter* colonization in Norwegian broiler flocks. Epidemiol Infect 1993; **111**: 245–55.
21. World Health Organization. Report on a WHO consultation on epidemiology and control of campylobateriosis. Geneva: World Health Organization, 1994; report no. WHO/CDS/VPH/94.135.
22. Schoeni JL, Doyle MP. Reduction of *Campylobacter jejuni* colonization of chicks by cecum-colonizing bacteria producing anti-*C. jejuni* metabolites. Appl Environ Microbiol 1992; **58**: 664–70.
23. Schoeni JL, Wong ACL. Inhibition of *Campylobacter jejuni* colonization in chicks by defined competative exclusion bacteria. Appl Environ Microbiol 1994; **60**: 1191–7.
24. Stern NJ. Mucosal competative exclusion to diminish colonization of chickens by *Campylobacter jejuni*. Poultry Science 1994; **73**: 402–7.
25. Stern NJ, Meinersmann RJ, Dickerson HW. Influence of antibody treatment of *Campylobacter jejuni* on the dose required to colonize chicks. Avian Dis 1990; **34**: 595–601.
26. Humphrey TJ, Henley A, Lanning DG. The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. Epidemiol Infect 1993; **110**: 601–7.
27. Oosterom J, Vereijken MJGM, Engels GB. *Campylobacter* isolation. Vet Quart 1981; **3**: 104.
28. Mazurier S, van de Giessen A, Heuvelman K, Wernars K. RAPD analysis of *Campylobacter* isolates: DNA fingerprinting without the need to purify DNA. Lett Appl Microbiol 1992; **14**: 260–2.
29. Hofstra H, van der Vossen JMBM, van der Plas J. Microbes in food processing technology. FEMS Microbiol Rev 1994; **15**: 175–83.
30. Plas J van der, Koster DS, Havekes WALM, *et al*. Restriction fragment length polymorphism (RFLP) typing of *Campylobacter* strains. In: Report on a WHO consultation on epidemiology and control of campylobacteriosis. Geneva: World Health Organization, 1994; WHO report no. WHO/CDS/VPH/94.135, 103–10.
31. Plas J van der, Hofstra H, Huis in 't Veld JHJ, *et al*. DNA probe assays for detection, identification and typing of *Campylobacter* species and *Helicobacter pylori*. Microb Ecol Health Dis 1993; **4**: S60.
32. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucl Acids Res 1991; **19**: 4008.
33. Giessen AW van de, Bloemberg PPM, Ritmeester WS, Tilburg JJHC. Epidemiological study on risk factors and risk reducing measures for campylobacter infections in Dutch broiler flocks. Epidemiol Infect 1996; **117**: 245–50.
34. Goossens H, Giesendorf BAJ, Vandamme P, *et al*. Investigation of an outbreak of *Campylobacter upsaliensis* in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotypic methods. J Infect Dis 1995; **172**: 1298–305.
35. Weijtens MJBM, Bijker PGH, van der Plas J, Urlings HAP, Biesheuvel MH. Prevalence of *Campylobacter* in pigs during fattening; an epidemiological study. Vet Quart 1993; **15**: 138–43.