

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

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(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 100 000 persons (2%), some 300 000 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

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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 10 000–25 000 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.

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 µm 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<sub>2</sub>, 10% CO<sub>2</sub> and 83% N<sub>2</sub>) 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 µl of distilled water. Then the suspension was heated to 100 °C for 3 min and centrifuged. The supernatant was adjusted to an O.D.<sub>260</sub> of 0.15 by dilution in distilled water and 5 µl 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

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	CATCTCCCTAGTCAAGCCT
JUN4	AAGATATGGCACTAGCAAGAC

photographed under UV-transillumination to visualize the RAPD pattern.

### 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 µl) had final concentrations of 20 mM Tris/HCl pH 8.3, 50 mM KCl, 3 mM MgCl<sub>2</sub>, 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 µl 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

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 cycle no.	Broiler house C1		Broiler house C2		Broiler house C3	
	RAPD type (no. of samples)	<i>Cam.</i> species (no. of isolates)	RAPD type (no. of samples)	<i>Cam.</i> species (no. of isolates)	RAPD type (no. of samples)	<i>Cam.</i> species (no. of isolates)
1	ND*		ND (10)		ND (10)	
2	A (10)	<i>C. jejuni</i> (2)	A (10)	<i>C. jejuni</i> (2)	A (5) B (2) ND (3)	<i>C. jejuni</i> (2) <i>C. coli</i> (2)
3	ND (10)		C (2) D (2) E (2) ND (4)	<i>C. jejuni</i> (2) <i>C. coli</i> (1) <i>C. coli</i> (1)	C (2) F (1) G (3) ND (4)	<i>C. jejuni</i> (2) <i>C. jejuni</i> (1) <i>C. jejuni</i> (3)
4	ND (10)		A (9) ND (1)	<i>C. jejuni</i> (2)	A (8) ND (2)	<i>C. jejuni</i> (1)
5	ND (10)		H (2) I (2) ND (6)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1)	J (2) K (2) ND (6)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1)
6	H (2) I (2) L (2) ND (4)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1) <i>C. coli</i> (1)	H (4) I (2) L (2) ND (2)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1) <i>C. coli</i> (1)	H (4) L (2) ND (4)	<i>C. jejuni</i> (1) <i>C. coli</i> (1)
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

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)	<i>C. coli</i> (1)	Parent flocks of broilers in house C1 and C2	6	AA (4)	<i>C. jejuni</i> (1)
		N (1)	<i>C. coli</i> (1)			AB (1)	<i>C. jejuni</i> (1)
	O (1)	<i>C. coli</i> (1)	AC (1)			<i>C. jejuni</i> (1)	
	P (1)	<i>C. coli</i> (1)	AD (1)			<i>C. coli</i> (1)	
	Q (1)	<i>C. coli</i> (1)	AE (1)			<i>C. jejuni</i> (1)	
	Q (1)	<i>C. coli</i> (1)	AF (5)			<i>C. coli</i> (1)	
	R (1)	<i>C. coli</i> (1)	AG (1)			NI†‡	
	S (3)	<i>C. coli</i> (3)	AH (1)			<i>C. jejuni</i> (1)	
	Q (4)	<i>C. coli</i> (4)	ND (5)				
	8	S (6)	<i>C. coli</i> (6)			CNT§ (45)	<i>C. jejuni</i> (1)
Broiler houses floors and walls	4	T (1)	<i>C. coli</i> (1)	ND (5)	7	ND (3)	
		U (3)	<i>C. coli</i> (3)	of broilers in house C3	6	ND (20)	
	5	V (2)	<i>C. coli</i> (2)	7	CNT (26)	<i>C. jejuni</i> (1)	
	8	W (1)	<i>C. coli</i> (1)		ND (4)		
	Dog	5	X (1)	<i>C. coli</i> (1)			
		5	Y (2)	<i>C. coli</i> (2)			
	Pigeons	7	Z (2)	<i>C. coli</i> (2)			
		7	ND† (3)				
	Beetles	4	ND (3)				
		8	ND (3)				
Pigeons	5	ND (1)					
	7	ND (2)					
Beetles	7	ND (2)					
	7	ND (2)					

\* 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 cycle no.	Broiler house D1		Broiler house D2	
	RAPD type (no. of samples)	<i>Cam.</i> species (no. of isolates)	RAPD type (no. of samples)	<i>Cam.</i> species (no. of isolates)
1	BA (3) BB (7)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1)	BB (10)	<i>C. jejuni</i> (1)
2	BA (1) BB (9)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (2)	BA (1) BB (9)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (2)
3	BB (10)	<i>C. jejuni</i> (1)	BA (9) BB (1)	<i>C. jejuni</i> (1) <i>C. jejuni</i> (1)
4	ND* (10)		BA'§ (10)	<i>C. jejuni</i> (1)
5	ND (10)		BA (1)	<i>C. jejuni</i> (1)
6	BB'† (10)	<i>C. jejuni</i> (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)	<i>Cam.</i> species (no. of isolates)
Cattle	2†	BB (4) ND‡ (6)	<i>C. jejuni</i> (3)
	4	ND (5)	
	6	BB (1) ND (12)	<i>C. jejuni</i> (1)
	7	CNT§ (1) ND (4)	<i>C. jejuni</i> (1)
		ND (1)	
Pony	4	ND (1)	
Dog	4	ND (3)	
Insects	5†	BA (1) ND (1)	<i>C. jejuni</i> (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) ND (3)	<i>C. jejuni</i> (1)
	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.

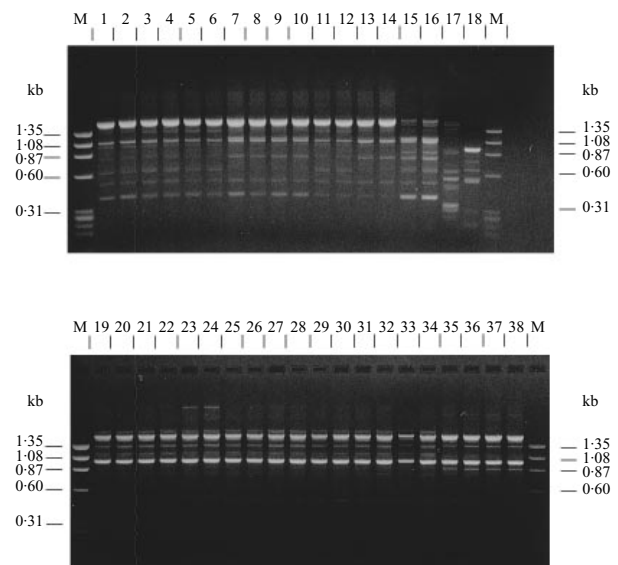
¶ RAPD-pattern BB' is only slightly different from pattern BB.

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



**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).

not sampled). Thus, the organism was isolated from flocks 3 and 6 in house D1 and from flocks 3–5 in house D2.

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 from 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 from the floors and walls of the empty broiler houses, feed, drinking water, the pony, the dog and the mouse.

## 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.

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