Routes for *Campylobacter* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse

L. HERMAN[1]*, M. HEYNDRICKX[1], K. GRIJSPEERDT[1],
D. VANDEKERCHOVE[2], I. ROLLIER[3] AND L. DE ZUTTER[4]

[1] *Agricultural Research Centre, Department of Animal Product Quality, Brusselsesteenweg 370, B-9090 Melle, Belgium*
[2] *Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Brussels, Belgium*
[3] *European Commission, Directorate General Health and Consumer Protection, Wetstraat 200, B-1049 Brussels, Belgium*
[4] *University of Ghent, Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke, Belgium*

SUMMARY

From April 1998 to March 2000, 18 broiler flocks were followed from the hatchery to the slaughterhouse. *Campylobacter* was not found in the hatchery, 1-day-old chicks or in the rearing house before the arrival of the chicks. The infection of broiler flocks increased continuously during the rearing time, with a total of seven positive flocks at the end of rearing. Farms with *Campylobacter*-positive broilers were characterized by the circulation of *Campylobacter* in the environment (puddles, dung hill) and on the footwear of the farmer. The administration of antibiotics did not significantly reduce *Campylobacter* shedding. With the exception of one flock during rearing and a few flocks in the slaughterhouse with a mixed *Campylobacter coli–Campylobacter jejuni* infection, *C. jejuni* exclusively was found both during rearing and on the carcasses. A significant correlation exits between the contamination of the broilers during rearing and the carcasses after processing. No slaughterhouse was able to avoid contamination of carcasses when status-positive animals were delivered. Moreover, six negatively delivered flocks yielded positive carcasses, the result of a supplementary contamination, which occurred during transport and slaughtering.

INTRODUCTION

Campylobacter jejuni and *C. coli* are the most common bacterial enteropathogens for humans in developed countries. During the last decade an increasing incidence of campylobacteriosis has been reported with *C. jejuni* as the most common *Campylobacter* species and *C. coli* accounting for approximately 5–10 % of the cases [1]. Eating and handling of contaminated poultry have been recognized as important risk factors for human infection [2–4].

The prevalence of *C. jejuni* in broiler flocks and broiler carcasses is very high and was estimated by a German study to be 45·9 and 43 %, respectively [5]. A Belgian study in 2000 showed a broiler carcass contamination rate of 33·9 % [6]. There is evidence that most *C. jejuni* and *C. coli* contaminations are introduced in broiler flocks by horizontal transmission during rearing rather than by vertical transmission from breeders to broilers [7–10]. Risk factors for the occurrence of *Campylobacter* in broiler flocks have been identified in several European countries as being due to the lack of appropriate hygiene barriers and infestation with insects and rodents [11, 12], animals

* Author for correspondence.

in the vicinity of the broiler house, livestock other than chickens on the farm, a down period of less than 14 days and dividing the flocks for slaughter [13]. In several European countries, an elevated *Campylobacter* occurrence in live broilers has also been observed during the summer months [11, 12, 14].

During the slaughter process an increase in carcass contamination is observed especially during evisceration where the potential exists for transferring intestinal microflora [15, 16]. Recent evidence indeed suggests that contaminated faeces escape from the cloaca during defeathering in the slaughterhouse, which leads to a higher broiler carcass contamination [17].

To implement a successful and cost-effective control programme for *Campylobacter*, knowledge about the relative contribution of risk factors in the whole poultry meat production chain is required. This article describes a quantitative epidemiological study of risk factors contributing to thermotolerant *Campylobacter* contamination of poultry meat in Belgium. For this, 18 individual broiler flocks were intensively studied from the hatchery to the slaughterhouse.

MATERIALS AND METHODS

Sample collection

During the period April 1998 to March 2000 a total of 18 broiler flocks, 16 independent and 2 successive flocks in the same house, were repeatedly sampled and analysed from the hatchery to the slaughterhouse. A wide range of samples was taken, as described in Table 1. Between 4 and 7 pools of 10 caecal drops were collected at each sampling day during rearing. Footwear of the farmer (used outside the broiler house, called 'dirty'; exclusively used inside the broiler house, called 'clean') was rinsed with 250 ml of buffered peptone water (BPW) (Oxoid Ltd, Basingstoke, UK) in large sterile plastic bags. Birds were about 42 days old when slaughtered. At the slaughterhouse the following samples were taken: 6 pools of faecal material from the transport containers, 6 pools of 10 caeca taken from the intestinal packages and 30–60 carcasses after refrigeration. All samples were put into plastic bags and boxes, cooled in an ice-box and immediately transported to the laboratory.

Thermotolerant *Campylobacter* analysis

After manual mixing of the samples, a loop from the pooled caecal drops, the faeces and the pooled caeca were directly streaked on the selective medium charcoal cefoperazone desoxycholate agar (CCDA), composed of *Campylobacter* blood-free selective agar base (no. CM739, Oxoid) and CCDA selective supplement (no. SR155, Oxoid), containing cefoperazone and amphotericin B. For the pooled caecal drops, another loop was streaked on CCDA after the addition of BPW (10 ml/g sample) and homogenization. All samples were also enriched for *Campylobacter* in Preston broth, composed of Nutrient Broth no. 2 (Oxoid), Preston *Campylobacter* selective supplement (SR117, Oxoid), containing polymyxin B, rifampicin, trimethoprim and cycloheximide, and 5% lysed horse blood. The following sample volumes were enriched: (1) samples collected at the farm: 5 ml or 5 g material in 45 ml Preston broth, 100 ml water filtered through a 0·22 μm filter and enriched in 20 ml Preston broth and (2) samples collected in the slaughterhouse: 1 ml of homogenized material in BPW in 9 ml Preston broth. After 24 and 48 h of micro-aerophilic (6% CO_2, 6% H_2, 4% O_2 and 84% N_2) incubation at 42 °C, a loop of the culture in Preston broth was streaked on CCDA. The CCDA plates were incubated at 42 °C for 24–48 h under micro-aerophilic conditions. Suspected colonies were microscopically investigated for the presence of spirals. A sample was determined positive for *Campylobacter* as soon as at least one of the isolation methods yielded a positive result.

Confirmation of presumptive *Campylobacter*

The bacterial cells were dissolved in 100 μl of H_2O and centrifuged for 2 min at 13 000 g. The pellet was resuspended in 100 μl of 0·05 M NaOH, 0·125% SDS and heated for 17 min at 90 °C. PCR was performed in a final volume of 50 μl containing 50 mM KCl, 10 mM Tris–HCl (pH 8·3), 1·5 mM $MgCl_2$, 0·5% Tween-20, 0·01% gelatine, 200 μM of each dNTP, 1·5 U of AmpliTaq DNA polymerase (PerkinElmer, Norwalk, CT, USA), 50 pmol of each primer and 1 μl of crude cell lysate. The mixture was subjected to 30 cycles of amplification in a thermal cycler (Cetus 9600; PerkinElmer). The first cycle was preceded by denaturation for 1 min at 95 °C. Each cycle consisted of denaturation for 15 s at 95 °C, annealing for 15 s at the indicated temperature (see below), and elongation for 30 s at 72 °C. A final elongation for 8 min at 72 °C followed the last cycle. The PCR products were analysed on a 1·5% (w/v) Seakem ME agarose gel (FMC Bioproducts, Rockland, ME, USA).

For identification of isolates on the *Campylobacter* genus level, the 16S rDNA-based primer pair C412F

(5′-GGATGACACTTTTCGGAGC-3′) and C1288R (5′-CATTGTAGCACGTGTGTC-3′) was used with an annealing temperature of 55 °C [18]. Identification of isolates as one of the thermotolerant campylobacters, *C. jejuni* or *C. coli*, was performed in a multiplex PCR at an annealing temperature of 63 °C with the aspartokinase gene-based primer pair COL3.3 (5′-GGTATGATTTCTACAAAGCGAG-3′) and COL4.4 (5′-ATAAAAGACTATCGTCGCGTG-3′) for *C. coli* and the hippuricase gene-based primer pair JEJ3.3 (5′-GAAGAGGGTTTGGGTGGTG-3′) and JEJ4.4 (5′-AGCTAGCTTCGCATAATAACTTG-3′) for *C. jejuni* described [19].

When no PCR identification could be achieved, the identity of the presumptive *Campylobacter* isolates was determined by sequence analysis of the 16S ribosomal DNA, amplified with the forward primer pA (5′-AGAGTTTGATCCTGGCTCAG-3′, position 8–27 on the *E. coli* sequence) and the reverse primer pH (5′-AAGGAGGTGATCCAGCCGCA-3′, position 1541–1522) at an annealing temperature of 59 °C. The PCR product was purified with the High Pure Product Purification kit (Boehringer–Mannheim, Mannheim, Germany) and the sequence was determined by the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) with the forward primers pA and PD′ (5′-CAGCAGCCGCGGTAATAC-3′, position 519–536) and the reverse primer PD (5′-GTATTACCGCGGCTGCTG-3′, position 536–519) and primer pH (BCCM/LMG Bacteria Culture Collection, Gent, Belgium, T. Coenye & B. Hoste, personal communication). After purification by NaOAc/ethanol precipitation, the sequence reactions were analysed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Identification was based on a FASTA search showing homology with known sequences of the EMBL database.

Determination of *Campylobacter* status

To determine the *Campylobacter* status of a flock, caecal drops were taken inside the rearing house at days 14, 28 and 42. If *Campylobacter* was detected in at least one of the 4–7 pools of 10 caecal drops at a minimum of one sampling time, the flock was considered positive for *Campylobacter*.

Analysis of potential risk factors for the flock status

More than fifty different sample sources were used to sample the broiler flocks, their housing facilities and environment for the presence of *Campylobacter* (Table 1). These were grouped in seven categories, to allow analysis of their significance in relation to the status of the flock: (*a*) hatchery, (*b*) animal material in the broiler house, days 1–42, (*c*) broiler house hygiene, day 1, (*d*) water in the broiler house, days 14–42, (*e*) movable material, i.e. 'vectors', easily transported into and out of the broiler house, days 1–42, (*f*) animal material in the environment, days 1–42, and (*g*) non-animal material in the environment, days 1–42. The influence of the use of antibiotics on the flock's status was also examined. The analysis was performed using unifactorial logistic regression, with 'status' as the dependent variable. The independent variables were continuous in the case of the sampling categories, which consisted of the proportion of samples from which *Campylobacter* had been isolated, or dichotomous, as was the case for the use of antibiotics. The effect of the use of antibiotic therapy on the proportion of *Campylobacter*-positive caecal droppings was tested using an ANOVA. Correlation between movable material, environmental samples, water in the house, faecal material from transport crates, and caeca after slaughter on the one hand, and caecal drops on the other hand, was assessed using Pearson's correlation. To obtain the confidence limits on the correlation coefficients, a non-parametric bootstrapping was done. The software used was SPSS 8.0 for Windows and S-Plus 4 for Windows.

Analysis of potential risk factors for carcass quality at the slaughter plant

Carcass quality was defined as the proportion of *Campylobacter*-contaminated carcasses after processing. The significance of *Campylobacter* isolation from caecal drops, transport crates' faecal material as well as caeca after slaughter in relation to carcass quality was tested using Pearson's correlation. The influence of the flock's status, the identity of the slaughter plant, the gut evisceration method, and the order in which the flocks were slaughtered, on carcass quality, was tested by ANOVA. The software used was SPSS 8.0 for Windows.

RESULTS

Campylobacter prevalence in the production chain of 18 flocks

A total of 18 broiler flocks, 16 independent and 2 successive flocks in the same broiler house (nos. 6 and 7),

Table 1. *Types of samples taken per category*

Category	Code	Samples taken
Hatchery	a	Incubators, valve water, bowels and yolk sacs of diseased or dead chicks
Animal material in broiler house (days 1–14–28–42)	b	Invertebrate animals, rodents, dung, feathers
Hygiene broiler house (day 1*)	c	Nipple water, chicks in walls or floors, wet straw or wood shavings
Water in broiler house (days 14–28–42†)	d	Nipple water, wet straw or wood shavings
Movable material (days 1–14–28–42)	e	Clean footwear, dirty footwear, wheelbarrows, bucket, cleaning material
Animal material environment (days 1–14–28–42)	f	Faecal material other domestic or wild animals, dung hills, spilled dung, overshoes or footwear in other stables, invertebrates, container with dead chickens, feathers
Non-animal material environment (days 1–14–28–42)	g	Disinfection tray at entrance stable, empty barrels, ditch water, pond water, puddles, household and garden refuse, grass silage, maize silage, wet bedding other stables, drinking water other animals, compost heap, overshoes, feed other animals, drains
Golden standard (days 14–24–42)	h	Caecal droppings
Faecal material crates	i	Faecal material in transport crates for broilers
Caeca	j	Caecal material from intestinal packages
Carcasses after processing	k	Neck skin after rapid chilling

* Day 1, the day of collection and transport of the 1-day-old broiler chicks from the hatchery to the broiler house.
† Days 14–28–42, sampling ages of the chicks during rearing.

were followed from the hatchery to the slaughter-house (Table 2), inclusive of sampling of the broiler house and the farm environment (Table 3, Fig. 1). The study included a total of 7 different hatcheries, 17 different poultry houses on 17 different farms and 9 different slaughterhouses. In the hatchery, and in the broiler house just before arrival of the 1-day-old chicks, no *Campylobacter* was isolated in any of the samples. The *Campylobacter* status was determined as positive by testing positive caecal drops in 7 of the 18 flocks. The amount of positive flocks increased during rearing from 3 flocks at 14 days, to 4 flocks at 28 days and 7 flocks at 42 days, just before slaughtering. In the two successive flocks, only the flock following secondly (flock no. 7) received a positive status.

In 12 flocks, a (sub)therapeutic treatment of antibiotics and/or chemotherapeutics was given during rearing. It concerned quinolones and/or fluoroquinolones in 5 flocks, ampicillin in 1 flock, a sulphonamide in 1 flock, a fluoroquinolone and trimethroprim + sulphonamide in 1 flock, a fluoroquinolone, sulphonamide and tetracycline in 1 flock, a macrolide, lincosamide and polypeptide antibiotic in 1 flock, a combination of a lincosamide with an aminoglycoside and a quinolone in 1 flock, and a combination of a lincosamide with an aminoglycoside in 1 flock.

In the environment of the broiler house (i.e. clean footwear in the hygiene gate and samples taken outside and in other broiler houses), a high contamination rate was detected with 11 positive farms out of the 16 for which this type of sample was taken. On six farms with *Campylobacter* status-negative broilers, *C. jejuni* was isolated from the environment outside the broiler house from the following samples: faecal material of wild animals (e.g. birds) found around the house (flock nos. 5 and 17), faecal material of poultry in other houses (flock nos. 10 and 13), dung hill (flock nos. 6 and 18), ditch water (flock no. 18) and puddles

Table 2. *Prevalence of* Campylobacter *in the production chain of 18 broiler flocks*

Flock/no. houses[a]	Hatchery	Rearing period on farm					Slaughtering phase			Slaughter-house[j]
		Antibio.[b]	Hygiene[c]	Environ.[d]	House[e]	Status[f]	Crates[g]	Caeca[h]	Carcass[i]	
1/1	n.d.[l]	—	0/5[k]	n.d.	0/10[k]	0/11[k]	0/6[k]	1/6[k]	11/60[k]	A*
2/1	n.d.	1×	0/1	0/1[k]	1/3	15/19	6/6	6/6	60/60	B
3/3	0/1[k]	2×	0/2	0/8	2/10	14/17	6/6	6/6	30/30	A*
4/1	0/1	2×	0/2	n.d.	0/7	0/15	2/6	0/6	0/30	B
5/1	n.d.	1×	0/3	1/29	0/5	0/16	0/6	0/6	0/60	A
6/3	n.d.	—	0/2	1/34	0/9	0/14	4/6	0/6	0/30	A
7/3	n.d.	—	0/4	4/28	1/5	9/13	6/6	6/6	17/30	A
8/3	0/4	3×	0/2	0/20	0/2	0/11	0/6	0/6	0/60	B*
9/3	0/1	—	0/3	5/19	1/4	3/16	6/6	6/6	60/60	B
10/8	n.d.	1×	0/5	2/23	0/9	0/19	6/6	6/6	47/47	C
11/1	0/1	1×	0/2	4/29	3/8	11/15	6/6	6/6	59/60	D
12/1	0/3	—	0/1	3/31	1/6	4/9	6/6	6/6	30/30	E
13/3	0/4	—	0/1	3/19	0/3	0/19	6/6	6/6	20/60	F
14/2	0/2	1×	0/1	0/26	0/4	0/10	n.d.	0/6	28/60	G*
15/5	0/1	4×	0/4	1/28	0/4	0/12	6/6	6/6	60/60	G
16/1	0/5	2×	0/2	0/26	0/6	3/27	6/6	6/6	30/30	H*
17/4	0/8	1×	0/3	1/31	0/5	0/15	5/6	2/6	1/30	I
18/1	0/2	1×	0/2	3/36	0/4	0/14	1/6	0/6	0/30	D
+/total (%)			0/45	28/388 (7·2%)	9/104 (8·7%)	59/272 (21·7%)	72/102 (70·6%)	63/108 (58·3%)	453/827 (55%)	
Flocks+	0+	12+	0+	11+	6+	7+	14+;	12+	13+	
Flocks−	6 n.d.	6−	18−	5−; 2 n.d.	12−	11−	3−; 1 n.d.	6−	5−	

[a] Flock number/number of poultry houses on the rearing farm; [b] number of times antibiotic administration during rearing; [c] hygiene broiler house on day 1; [d] animal and non-animal material environment and movable material; [e] water and animal (except chicken caecal drops) material in broiler house; [f] pools of caecal drops; [g] faecal material from transport crates; [h] caecal material from intestinal packages; [i] neck skin from carcasses after rapid chilling; [j] asterisk after slaughterhouse identity code means flock not slaughtered first on the day; [k] number of *Campylobacter*-positive samples on the total number of investigated samples; [l] n.d., not determined.

Table 3. *Distribution of* Campylobacter-*positive samples* (*indicated in bold*) *taken inside the broiler house, in the hygiene gates and in the environment outside the broiler house from 7* Campylobacter-*negative and 6* Campylobacter-*positive flocks. The remaining 5 flocks did not yield positive samples from these sites* (*data not shown*). *Likewise, other environmental sample types* (*see Table 1*), *which were never found* Campylobacter-*positive, are not mentioned in this table*

Sample	Flock number with negative status							Flock number with positive status					
	5	6	10	13	15	17	18	2	3	7	9	11	12
In broiler house													
Nipple water (days 14–42)[d]	0/3[a]	0/3	0/3	0/3	0/3	0/3	0/3	**1/3**[f]	**1/3**	**1/3**	**1/3**	**2/6**	**1/4**
Wet straw or wood shavings	—[b]	0/2	0/1	0/1	—	0/1	—	—	**1/1**	0/1	0/1	0/1	—
Hygiene gate													
Clean footwear (days 14–42)	0/5	0/3	0/2	0/3	0/3	0/3	0/3	—	0/1	**1/3**	**1/2**	**2/3**	**1/6**
Outside broiler house													
Dirty footwear (days 14–42)	—	0/2	0/1	0/1	0/1	—	0/3	—	—	—	**1/1**	0/1	—
Faecal material wild animals (days 14–42)	**1/5**	0/5	0/2	—	0/1	**1/2**	0/1	—	0/3	**1/1**	0/3	0/1	0/5
Faecal material domestic animals (day 1)[c]	—	—	0/1	0/2	**1/2**[e]	0/1	0/3	—	—	—	0/1	0/1	0/1
Faecal material domestic animals (not poultry) (days 14–42)	0/4	0/4	0/1	0/2	0/3	0/3	0/4	0/3	—	—	0/2	0/3	**1/4**[e]
Faecal material from other poultry houses (days 14–42)	—	—	**2/10**	**1/2**	0/9	0/9	—	—	—	**1/8**	**2/2**	—	—
Dung hill (day 1)	—	**1/2**	—	—	—	—	**2/2**	—	—	—	—	0/2	0/1
Dung hill (days 14–42)	0/2	0/5	—	—	—	0/1	0/6	—	—	**1/4**	0/1	0/2	0/4
Ditch water (day 1)	—	—	—	—	—	0/1	**1/1**	—	—	—	—	—	—
Ditch water (days 14–42)	—	0/1	—	—	0/1	0/1	—	—	—	**1/2**	—	—	—
Puddles (days 14–42)	0/3	0/2	—	**2/2**	—	0/4	0/3	—	—	0/3	**1/2**	**1/3**	**1/4**
Container or bucket with dead chickens (days 14–42)	0/3	0/3	—	0/1	0/1	—	—	—	—	0/3	—	**1/4**	—

[a] Number of positive samples/total number of samples taken. Unless otherwise indicated, all positive samples yielded only *C. jejuni*; [b] sample not taken or not available in this flock; [c] sample taken at day 1 before arrival of 1-day-old chicks; [d] sample taken during rearing period; [e] *Campylobacter hyointestinalis*; [f] *Campylobacter coli*.

on the farm (flock no. 13) (Table 3). Flock no. 16 was the only flock with *Campylobacter*-positive animals where only caecal drops were found positive. It concerned a small fraction of the caecal drops at the last sampling day just before slaughter (3 positive samples out of 27). In 5 of the 6 flocks with *Campylobacter*-positive status, the nipple water inside the broiler house frequently tested positive for *C. jejuni* (on average 1 out of 3 samples), in 1 flock (flock no. 3), the wet straw or wood shavings under the nipples also tested positive. As could be expected, the clean footwear used for flock visits by the farmer was positive for *C. jejuni* in 4 of the 7 *Campylobacter* status-positive flocks. Furthermore, on these farms *C. jejuni* was most frequently isolated in the environment from the puddles (flock nos. 9, 11 and 12), followed by the faecal material from other poultry houses (flock nos. 7 and 9), the dirty footwear used outside the broiler houses (flock no. 9), the faecal material of wild animals (flock no. 7), the dung hill (flock no. 7), the ditch water (flock no. 7) and the container with dead chickens (flock no. 11).

With the exception of flock no. 2, *C. jejuni* exclusively was isolated from the caecal drops of the chickens. In flock no. 2, *C. jejuni* was isolated at sampling day 14 from the caecal drops, while at sampling day 28 no *Campylobacter* was isolated, probably because of antibiotic administration. At sampling day 42, *C. coli* was isolated, probably a cross-contamination from the pigs, which were reared on the same farm, although we were not able to isolate this species from the pig faeces at the time of sampling. In flock nos. 12 and 15, *C. hyointestinalis* was isolated from the faecal samples of the cows on the farms. *C. hyointestinalis*
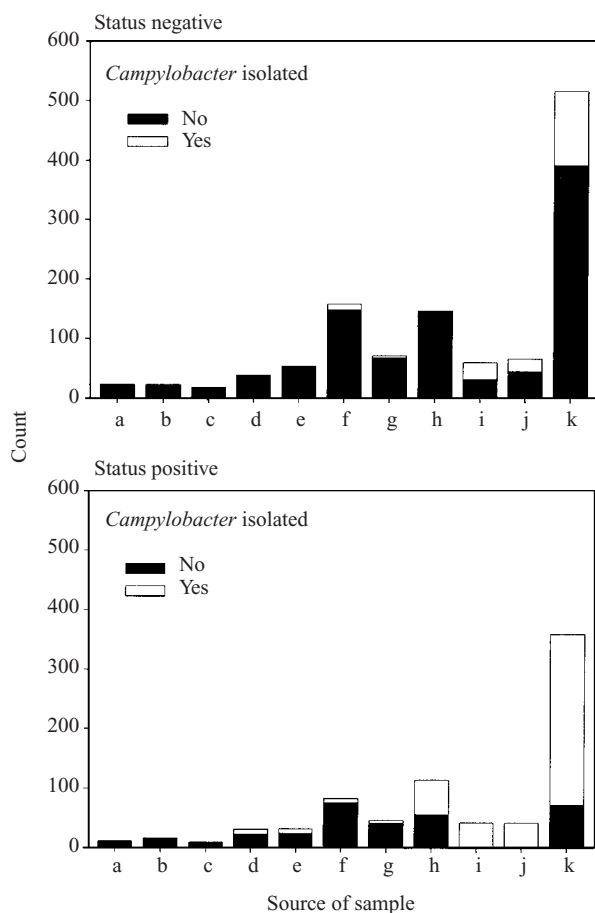
Fig. 1. Isolation of *Campylobacter* per sampling category, split according to the status of the flock. The codes in the *x* axis refer to Table 1.

was not isolated in any other sample taken at both farms. The faeces in the crates after transport of the broilers to the slaughterhouse could be sampled in 17 of the 18 flocks. For 14 flocks, the faeces in the crates was found *Campylobacter* positive: 7 of these flocks were already positive during rearing, while the other 7 flocks were negative during rearing.

For 12 of the 18 flocks, it was arranged for the flock to be slaughtered first during the slaughtering day. After slaughtering, *Campylobacter* was isolated from the neck skin of the carcasses in 13 flocks and from the caecal content in 12 flocks. The 7 positive flocks during rearing all resulted in positive caeca and positive neck skin of the carcasses. In 3 flocks, which were *Campylobacter* negative during rearing and with positive faeces in the transport crates, no *Campylobacter* was isolated from the caeca nor the neck skin of the carcasses. Four flocks, however, which were *Campylobacter* negative during rearing and with positive faeces in the transport crates, were positive in the caeca

and on the neck skin of the carcasses. From flock no. 14, *Campylobacter* was isolated from the neck skin of the carcasses although no *Campylobacter* was found in any of the samples taken during rearing and in the caeca after slaughter. At the end of the slaughter process, 453 of the 827 carcasses tested were positive (55%). With the exception of flock nos. 2, 3, 7 and 13 *C. jejuni* was exclusively isolated from the faeces in the crates, the caeca and the neck skin of the carcasses (Table 4). In flock no. 2 *C. coli*, isolated from the caecal drops at the end of the rearing period of the broilers, was isolated from the faeces of the crates after transport and in the caeca. On the carcasses, however, both *C. coli* and *C. jejuni* were isolated. In flock no. 3, where the crates and caeca were contaminated with *C. jejuni*, the neck skin of the carcasses showed a mixed infection with *C. coli*. In flock nos. 7 and 13, a mixed infection of *C. jejuni* and *C. coli* was seen in the crates and the caeca, respectively. These results clearly show a cross-contamination during transport of the broilers to the slaughterhouse and during the slaughter process.

Determining factors for *Campylobacter* contamination of broilers at the farm

Only on farms with *Campylobacter*-positive broilers was *Campylobacter* isolated from the movable material on the farm and from the water in the broiler house compared to farms with *Campylobacter*-negative broilers (Fig. 1). Only four of the seven categories of samples tested were used in the risk factor analysis, because the results for hatchery, animal material in the broiler house, and broiler house hygiene, were all negative (no *Campylobacter* isolation). The unifactorial logistic regression indicates that the movable material on the farm and the (drinking) water in the broiler house are significant risk factors for the contamination of the broilers (Table 5). Using the bootstrapped Pearson's correlation, a significant relation was found between the contamination rate of the broilers (i.e. proportion of positive caecal droppings) and the presence of *Campylobacter* in movable material as well as water sampled in the broiler house (Table 6). In light of the very high value of the latter correlation, this contaminated (drinking) water is probably an important risk factor for spreading *Campylobacter* to the other animals of the flock. Antibiotic administration during rearing of broilers did not reveal any significant effect on the status (Table 5) or the amount of

Table 4. *Isolation of* C. coli *and* C. jejuni *in samples from the transport and slaughter phase of broilers*

Flock no.[a]	Crates[b]		Caeca[c]		Carcasses[d]	
	C. coli	C. jejuni	C. coli	C. jejuni	C. coli	C. jejuni
Flocks with negative status during rearing (%)						
1	—[e]	—	n.d.[e]	n.d.	0	100
4	0	100	—	—	—	—
6	0	100	—	—	—	—
10	0	100	0	100	0	100
13	0	100	20	80	0	100
15	0	100	0	100	0	100
17	0	100	0	100	n.d.[f]	n.d.
Flocks with positive status during rearing (%)						
2	100	0	100	0	30	70
3	0	100	0	100	20	80
7	40	60	0	100	0	100
9	0	100	0	100	0	100
11	0	100	0	100	0	100
12	0	100	0	100	0	100
16	0	100	0	100	0	100

[a] Flocks with no positive *Campylobacter* sample and those from which the *Campylobacter* strains were not identified to species level are not mentioned in this table. In all flocks, with the exception of flock no. 2, *C. jejuni* was isolated during rearing. In flock no. 2 a contamination with *C. jejuni* was followed by *C. coli* during the last period of rearing; [b] faecal material from transport crates; [c] caecal material from intestinal packages; [d] neck skin from carcasses after rapid chilling; [e] no *Campylobacter* isolated; [f] n.d., not determined.

Table 5. *Unifactorial logistic regression with status flock as dependent variable*

Dependent variable	Independent variable	Type of variable	$P \chi^2$
Status flock	Water broiler house	Continuous	<0·001*
Status flock	Movable material	Continuous	0·002*
Status flock	Animal material environment	Continuous	0·648
Status flock	Non-animal material environment	Continuous	0·322
Status flock	Use of antibiotics	Dichotomous	0·387

* Highly significant.

Campylobacter-positive caecal drops that were found in positive flocks (Table 7).

Determining factors for *Campylobacter* contamination on broiler carcasses

A significant correlation was found between the contamination rate of the broilers and the faecal material from the transport crates to the slaughterhouse (Table 6). The correlation of the *Campylobacter* contamination rate of carcasses with the contamination of the animals during rearing is significant as indicated by the correlation analysis and highly significant as shown by ANOVA (Tables 6 and 7). During the broilers' transport to the slaughterhouse, an extra contamination occurs, as is seen from the positive faecal material from crates transporting status-negative animals (Fig. 1). The presence of *Campylobacter* in faecal material in transport crates is strongly correlated with the contamination rate of the chicken carcasses (Table 6). The same extra contamination of status-negative broilers during transport and/or

Table 6. *Bootstrapped Pearson's correlations for* Campylobacter *contamination rate of broilers* (caecal drops) *and carcasses* (neck skin). *If the 95 % CI does not contain 0, then the correlation is statistically significant*

Variable 1	Variable 2	r^2	95 % CI
Positive broilers (%)	Movable material	0·64	0·14–0·95
Positive broilers (%)	Animal material from environment	0·024	−0·46–0·48
Positive broilers (%)	Non-animal material from environment	0·13	−0·22–0·79
Positive broilers (%)	Water in broiler house days 14–42	0·97	0·94–0·99
Positive broilers (%)	Faecal material from crates	0·51	0·33–0·77
Positive broilers (%)	Caeca after slaughter	0·60	0·35–0·81
Positive broilers (%)	Positive carcasses (%)	0·60	0·29–0·82
Positive carcasses (%)	Faecal material from crates	0·79	0·62–0·92
Positive carcasses (%)	Caeca after slaughtering	0·88	0·67–0·96

Table 7. *ANOVA results for* Campylobacter *contamination of broilers* (caecal drops) *and carcasses* (neck skin)

Dependent variable	Independent variable	P
Positive broilers (%)	Use of antibiotics (yes/no)	0·24
Positive carcasses (%)	Status flock	<0·001*
Positive carcasses (%)	Slaughterhouse identity	0·78
Positive carcasses (%)	Gut evisceration method	0·59
Positive carcasses (%)	First slaughtered or not	0·99

* Highly significant correlation.

slaughtering can also be deduced from the isolation of *Campylobacter* from caeca taken after slaughter of status-negative broilers (Fig. 1). The contamination of caeca after slaughter is significantly correlated with the contamination rate of the chicken carcasses (Table 6).

The *Campylobacter* contamination rate of the broiler carcasses is not significantly correlated with the gut evisceration method (bowels suspended separately, bowels suspended on the back of the carcass, bowels put on tray) (Table 7). Slaughtering a flock first during the slaughtering day did not influence the *Campylobacter* contamination rate significantly (Table 7). Although the effect is not statistically significant (Table 7), some influence of the slaughterhouse identity was seen on the amount of positive carcasses (Fig. 2). Four of the seven slaughterhouses, which received status-negative broilers, were able to deliver them all or almost all as negative carcasses; while in two other slaughterhouses, all or nearly all the carcasses were contaminated. On the other hand,

all *Campylobacter*-positive animals resulted in at least 86·7% positive carcasses in all five different slaughterhouses, which received status-positive animals.

DISCUSSION

Our study confirms the low importance of the first steps in the broiler production chain for the contamination with *Campylobacter*. No *Campylobacter* strains were isolated from the samples taken at the hatchery and in the house before the arrival of the 1-day-old chicks. Similarly, Evans & Sayers [20] did not find any evidence of (environmental) survival of *Campylobacter* in broiler houses after adequate cleaning and disinfection.

We found a *Campylobacter* contamination frequency of 39% in live broiler flocks. This frequency is similar to reports from Sweden (27%) [11] and Denmark (43%) [14], but lower than reported for France (79·2%) [21]. The *Campylobacter* contamination in the 18 flocks of this study gradually increased
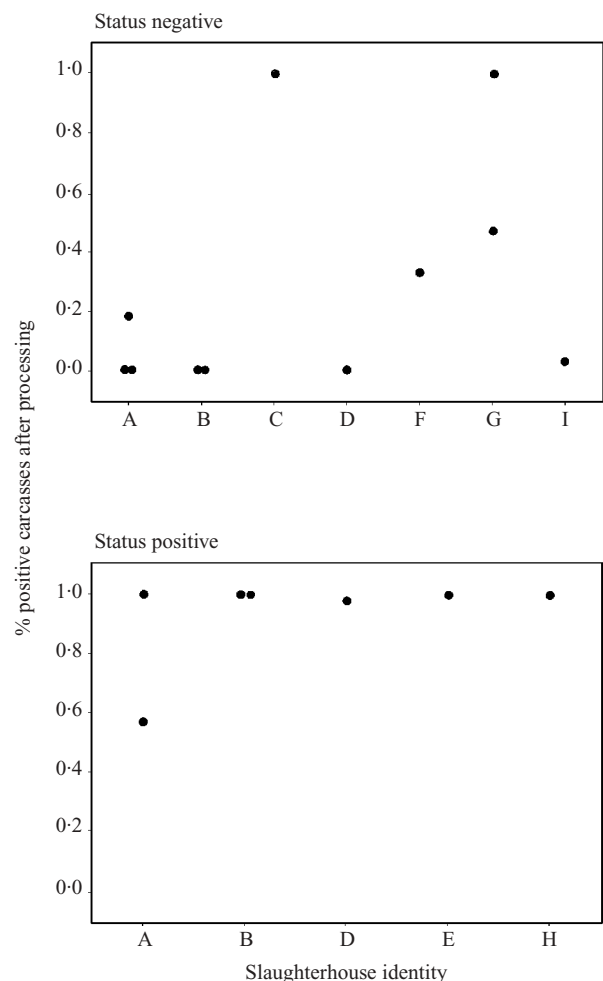
Fig. 2. *Campylobacter* contamination of carcasses in function of the identity of the slaughterhouse when status-negative or status-positive animals were delivered.

the spring and summer months, we judged our data to be too limited in this respect to find a seasonal effect in *Campylobacter* contamination as reported in the literature (e.g. Wedderkopp et al. [14]).

On farms with infected broilers, *Campylobacter* was isolated from domestic pigs, workers' boots and wild birds [23, 24]. Our study showed that the presence of *Campylobacter* in other animals does not automatically correlate with positive broiler flocks. Farms with *Campylobacter*-positive flocks are characterized by a further circulation of *Campylobacter* in the environment (e.g. in puddles) and on the movable material (e.g. the footwear of the farmer) of the farm. This indicates the importance for the correct use of the hygiene gate and the decontamination of footwear. Humphrey et al. [25] also reported the importance of dipping boots in disinfectant before entering the broiler houses. Similar conclusions were also obtained from an extensive 2-year study of Danish broiler flocks, in which no significant reductive effects of intensive cleaning and disinfection procedures combined with an extended period of empty housing could be demonstrated on the *Campylobacter* infection of poultry [14]. As already stated above, once the contamination enters the house, the drinking water functions as an important vector for further spreading of the contamination in the flock. Therapeutic antibiotic administration, which occurred in 12 of the 18 flocks, had no significantly reducing effect on the *Campylobacter* shedding in caecal drops. Also the antibiotic broiler-feed additives flavophospholipol (FPL; Flavomycin, bambermycins) and salinomycin sodium (SAL; Sacox) did not affect the incidence or the degree of *Campylobacter* shedding [26]. This is in contrast to *Salmonella* where a pronounced effect on shedding was observed by antibiotic treatment of the broiler flocks [27].

The *Campylobacter* contamination during rearing is significantly correlated with the contamination of the final product. However, an extra *Campylobacter* contamination occurred during transport and the slaughter process. The amount of positive flocks increased from 7 during rearing to 13 for the chicken carcasses leaving the slaughterhouse. Moreover, for 2 flocks with an exclusive *C. jejuni* contamination during rearing, an additional contamination with *C. coli* was observed either in the transport crates or on the carcasses. Also in the United Kingdom, it was observed that carcasses from *Campylobacter*-negative flocks reaching the abattoir were contaminated during processing [28]. This extra contamination may

from three flocks (17%) at 2 weeks of rearing till seven flocks (39%) at 6 weeks. This gradual increase of infection was also reported by Evans & Sayers [20] where more than 40% of the flocks were infected with *Campylobacter* by the time the chicks were 4 weeks old and over 90% by 7 weeks. This gradual increase of *Campylobacter* infection is probably due to an increased risk for *Campylobacter* transmission into the broiler house. This increased challenge can be mediated through contaminated drinking water, which was shown here to be an important vector for spreading *Campylobacter* contamination in the broiler house. Colonization of broiler chicks with *Campylobacter* is reported to occur independently of chick age and rapidly after challenge with the pathogen [22]. As a consequence the *Campylobacter* status of a flock can best be determined just before slaughtering. Although most of the positive flocks were found in

originate from the abattoir-processing environment, but evidence was also found for contaminated transport crates entering the poultry farms as a possible source [29]. In our study, faeces from transport containers were contaminated in 14 flocks, which indicates insufficient cleaning and disinfection of the containers. The extra contamination observed in the crates after transport could be traced back in the caeca of the slaughtered chickens, which were positive in a total of 12 flocks. Also catching of the birds has been reported as a high risk factor for introduction of *Campylobacter* in the flock just before slaughter [30]. These results suggest a quick colonization of the broiler intestine, which was also reported by Achen et al. [31] who showed that, as soon as 24 h after oral inoculation, 50% of the broilers were shedding *Campylobacter*.

The evisceration method and the time of slaughter (slaughtered first during the day or not) did not have a significant influence on the contamination of the final product. Although our results did not indicate a statistically significant influence, the identity of the slaughterhouse seemed to play an important role in the *Campylobacter* contamination rate of the carcasses, which indicates the importance of hygiene during slaughter. However, no slaughterhouse was able to avoid positive carcasses when status-positive animals were delivered. Therefore, a programme to obtain *Campylobacter*-free chicken carcasses has to decrease the degree of contamination in the different levels of the broiler production chain, i.e. during rearing on the farm, transport to the slaughterhouse and during carcass processing.

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