Some potential sources for transmission of *Campylobacter jejuni* to broiler chickens

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ABSTRACT

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Aims: The aim of the study was to determine *Campylobacter jejuni* contamination and prevalence on fomites moving between broiler farms and the processing plant in the period after cleaning and before departure to harvest chickens. In addition, changes in the proportion of contaminated fomites in the course of a day were assessed.

Methods and Results: Pooled swab samples were obtained from pallets, crates, wheels of trucks, tractors and forklifts, truck beds, and from drivers' and catchers' boots. After enrichment in Bolton's broth *Campylobacter* were recovered on modified blood-free *Campylobacter* selective agar (mCCDA). Isolates were identified using tests for phenotypic and biochemical characteristics. Of the 209 samples collected, 53% were positive for *C. jejuni*, with all fomites positive except tractor wheels. Pallets had the highest contamination rate at 75%. More than 50% of catchers' boots, drivers' boots, crates and truck wheels were positive. Forty-seven per cent and 31% of truck beds and forklift wheels, respectively, were contaminated. The proportion of contaminated fomites did not change significantly during the day.

Conclusions: This study has identified trucks, forklifts, pallets, crates, drivers' and catchers' boots as potential sources of *C. jejuni* for broilers.

Significance and Impact of the Study: *Campylobacter jejuni* contamination of broiler processing plant fomites was found to be extensive ranging from 31% for truck beds to 75% for pallets. The proportion of contaminated fomites was observed to be similar throughout the day. The impact of contaminated fomites as sources of colonization of broilers with *C. jejuni* is discussed.

Keywords: broilers, *Campylobacter*, colonization, fomites, prevalence.

INTRODUCTION

Poultry meat has been established as an important source of human infection with both *Campylobacter jejuni* and *C. coli* (Skirrow 1991; Pearson *et al.* 1996). There is a general consensus that a significant reduction in human infections can be achieved by reducing *Campylobacter* infection in broiler flocks (Lindblom *et al.* 1986; Evans 1992; van de Giessen *et al.* 1998). Consequently many investigations have

been carried out to identify transmission paths for *C. jejuni* colonization of broiler flocks in their sheds.

Findings by Doyle (1984), Lindblom *et al.* (1986) and Pearson *et al.* (1996) indicate that vertical transmission of *C. jejuni* from the parent flock to chicks is highly unlikely under natural conditions. Horizontal transmission is known to occur and important potential sources include poultry sheds, water, litter, feed, fauna and footwear (Clark and Bueschkens 1988; Kazwala *et al.* 1990, 1992; Shanker *et al.* 1990; Pearson *et al.* 1993). A combination of intervention measures targeting common sources of infection have been reported to prevent or reduce colonization in broilers or to prolong the time before infection (Kazwala *et al.* 1992; van de Giessen *et al.* 1998). Jacobs-Reitsma *et al.* (1995) observed that

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Campylobacter-positive and *Campylobacter*-negative broiler flocks could be housed on commercial farms at the same time.

Campylobacter jejuni spreads rapidly through a susceptible broiler flock following introduction (Stern 1992; Cawthraw *et al.* 1996). Experimental studies indicate that the spread from an infected bird to susceptible birds occurs within 72 h (Clark and Bueschkens 1988; Shanker *et al.* 1990) and that in large flocks of about 20 000 birds, the spread is logarithmic in nature (Montrose *et al.* 1985). Smitherman *et al.* (1984) showed that all the chickens in a flock became positive within 1 week of exposure. *Campylobacter jejuni* could be introduced to *Campylobacter*-free flocks at the first depopulation by equipment and personnel involved in the catching process. The breach in biosecurity that occurs during the catching operation also increases the risk of *C. jejuni* spread to other flocks on the farm.

An investigation was carried out to determine if foot wear and equipment used in the catching process were contaminated with *C. jejuni* following cleaning at the processing plant, to determine the proportion of contaminated fomites and to assess if the proportion of *C. jejuni*-contaminated fomites changes with time during the day.

MATERIALS AND METHODS

Sampling

Pallets, crates, truck beds, truck wheels and the drivers' boots were sampled before they departed from the processing plant, prior to each depopulation trip and just after washing. Catchers' boots were sampled in the morning before the catchers left for the first farm. The tractors made one trip each day between the plant and the farms and they were sampled before they left. Forklifts made one or two trips a day and they were sampled on each occasion.

To collect samples, a 5-cm^2 sterile metal template was pressed against the surface to be sampled and a cotton-tipped swab (Fort Richards, Auckland, New Zealand) moistened with buffered peptone water was rubbed vigorously over the entire area exposed by the template. A second dry swab was rubbed over the area in the same manner. The swabs were both broken below the handling area into a universal bottle containing 15 ml of Bolton's enrichment broth that had been prepared according to the manufacturer's instructions using *Campylobacter* enrichment broth (Lab M, Lab 135; Fort Richards), Bolton's supplement (Lab M, supplement X131; Fort Richard) and 5% (v/v) lysed horse blood (Life Technologies, Auckland, New Zealand). Each pallet was sampled from the floor of one of its rectangular forklift holes. The hole on the right was sampled each time. An inverted crate, used to hold chickens, was sampled on the bottom left corner. Each truck bed was divided into eight equal sections numbered one to eight and

four randomly selected sections were then sampled at the lateral rear corner. Eight wheels (from either 20- or 22-wheeled trucks) were selected randomly and sampled from the surface that contacted the ground. Three of the four tractor wheels were similarly selected and sampled. All forklift wheels (three) were sampled. Samples from the boots of the drivers and catchers were taken from the sole of the left heel. The number of fomites constituting a single-pooled sample was 3, 6, 1, 8, 3, 3, 1 and 6 for pallets, crates, truck bed, truck wheels, forklift wheels, tractor wheels, drivers' boots and catchers' boots, respectively.

Sampling occurred over a 6-day period. Each day, batches of samples were taken on six consecutive depopulation trips, occurring in the periods 05.30–06.30 hours, 06.30–07.30 hours, 07.30–08.30 hours, 09.30–10.30 hours, 10.30–11.30 hours and 11.30–12.30 hours. For all the depopulation trips samples were collected from pallets, crates, truck bed, truck wheels and drivers' boots (Table 2). One sample from a driver's boot was missed on the first day of sampling. Tractors, forklifts and catchers' boots were excluded from the sample batches (Table 2), as they were part of depopulation trips only once or twice per day.

Isolation and identification of *C. jejuni*

Swabs were incubated in Bolton's selective enrichment broth, within 2 h of collection, at 42°C for 48 h under microaerophilic conditions obtained using the Campy Gen^{TM} gas generating system (CN 25; Oxoid, Hampshire, UK). After incubation, sterile swabs were used to inoculate the broth onto modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid CM739 and SR155E) plates, which were incubated at 37°C under microaerophilic conditions for a further 48 h. Individual colonies characteristic of *Campylobacter* were then subcultured onto tryptic soya agar (TSA; Fort Richards) to obtain pure cultures and two discs, one containing 30 μg nalidixic acid (NA; Oxoid) and the other containing 30 μg cephalothin (KF; Oxoid) were placed on the TSA plates for antibiotic sensitivity testing. *Campylobacter jejuni* was identified using the following criteria: sensitivity to nalidixic acid, resistance to cephalothin, positive reaction for hippurate hydrolysis using hippurate discs (BBL^{TM} TAXO^{TM}; Becton Dickinson and Co., St Louis, MO, USA), and positive reactions to catalase and oxidase tests. Gram stains of isolates were examined and Gram-negative curved rods or cocci were considered characteristic of *Campylobacter*. Isolates were also examined using a dark field microscope for characteristic motility.

RESULTS

Campylobacter jejuni was isolated from all sampled sites except tractor wheels (Table 1). The pallets had the highest

Table 1 Isolation rates of *Campylobacter jejuni* from broiler processing plant fomites

Source	No. of pooled samples	No. of positives	Positives (%)
Pallets	36	27	75
Crates	36	21	58·3
Truck bed	36	17	47·2
Truck wheels	36	18	50
Drivers' boots	35	19	54·3
Catchers' boots	6	4	66·7
Forklift wheels	16	5	31·3
Tractor wheels	8	0	0
Totals	209	111	53·1

isolation rate of 75%. More than 50% of catchers' boots, drivers' boots, crates and truck wheels were positive. These fomites (excluding tractor wheels) had an average isolation rate of 57%.

The percentage of positive samples was lowest for batch number 1 and highest for batch number 5 (Table 2). On two occasions (5th batch on day 3 and 4th batch on day 4) *C. jejuni* was isolated from all potential sources of infection. In the 3rd batch on day 6, all the potential sources were negative for the organism (Table 2). More samples were positive for *C. jejuni* subsequent to the first batch on all days (Table 2). However, there was no statistically significant difference in the proportion of *C. jejuni*-contaminated fomites at different times of the day (Table 2).

DISCUSSION

Studies to identify fomites commonly contaminated with *C. jejuni* have previously concentrated on the poultry farm and within the poultry processing plant (Shanker *et al.* 1986; Evans 1992; Jacobs-Reitsma *et al.* 1995; Stern *et al.* 1995). On the farm, *C. jejuni* contaminates the environment once the chickens become colonized (Lindblom *et al.* 1986;

Gregory *et al.* 1997). At the poultry processing plant, the organism has been isolated from a number of critical control points (Wempe *et al.* 1983; Genigeorgis *et al.* 1986). Previously, only a few attempts were made to isolate the organism from fomites that enter poultry farms during depopulation. In this study, pallets, crates, trucks, tractors, forklifts, and the footwear of drivers and catchers were shown to be contaminated with *C. jejuni*. The fomites were sampled after cleaning but before they left for the farm, which strongly suggests that they would still have been contaminated with viable *C. jejuni* when they reached the farms. They can therefore be regarded as potential sources of infection for broiler chickens.

Tractor wheels were sampled in the morning before the tractors left for the farms. They were returned and washed at the end of each day. The absence of *C. jejuni* on tractor wheels is difficult to explain given the high contamination rate of the other fomites, but could be due to a number of factors including more effective cleaning with chlorinated water, death of *C. jejuni* from overnight exposure to high oxygen tension in the atmosphere or the transformation of the organism to a viable but nonculturable state (Rollins and Colwell 1986). In terms of transmission of *C. jejuni*, the tractors could still contribute as they were reportedly moved between farms during the day without being washed or disinfected.

All the fomites sampled were visibly contaminated with dirt and faecal material at the time of sampling and were usually wet unless they had dried overnight or over the weekend. *Campylobacter jejuni* may be protected from desiccation by faecal material and a wet environment (Rollins and Colwell 1986). Pallets were observed to have more dirt and faecal material than other equipment.

With a mean *C. jejuni* isolation rate of 57%, pallets, crates, drivers' boots, catchers' boots and forklift wheels, should be considered important potential vehicles for transmission of the organism to broilers. This study confirms the findings of van de Giessen *et al.* (1998) who isolated *C. jejuni* from

Table 2 Isolation rates of *Campylobacter jejuni* from broiler processing plant fomites at different time periods during the day

Batch no. (sampling period)	Number of positive samples (n = 5*)						Sum of positives (n = 30)	Positives (%)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6		
1 (0530–0630)	3†	2	3	1	2	2	13‡	44·8
2 (0630–0730)	4	2	4	4	2	3	19	63·3
3 (0730–0830)	4	4	4	3	3	0	18	60·0
4 (0930–1030)	2	1	3	5	3	2	16	53·3
5 (1030–1130)	4	3	5	3	2	3	20	66·7
6 (1130–1230)	1	4	3	3	3	2	16	53·3

*Samples were from five types of fomites:s pallets, crates, truck bed, truck wheels and drivers' boots.
†n = 4.
‡n = 29.

slaughterhouse crates, trucks and truck tires in The Netherlands.

The epidemiological significance of *C. jejuni* on footwear, vehicles and other equipment is that these fomites may be responsible for the introduction of the bacteria into susceptible flocks during the first depopulation. Fomites used in the sheds that are likely to cause direct transmission of *C. jejuni* into the sheds, are pallets, crates, forklifts, tractors and catchers' boots. Trucks and drivers are only capable of transmission to the farm environment, as they do not go into the sheds. Contaminated trucks and crates may also transmit *C. jejuni* to broilers during transport to the abattoir (Stern *et al.* 1995). In New Zealand, it is common practice for flocks to undergo two or more depopulations, and *C. jejuni* may spread amongst the birds remaining in the shed or even to other sheds in the farm, if it is introduced via the sources identified in this study. Although evidence to support this theory has not yet been reported, findings by others indicate that multiple depopulations increase the prevalence of *Campylobacter* in flocks (Berndtson *et al.* 1996; Hald *et al.* 2000). Hald *et al.* (2000) found that 50% of flocks that were slaughtered in a single depopulation were positive for thermophilic campylobacters, but that this prevalence rose to 85% if flocks were exposed to abattoir equipment and personnel at a prior depopulation. In another study, Wedderkopp *et al.* (2000) found a single depopulation flock prevalence of 41% and a prevalence of 100% for flocks slaughtered in six depopulations. Slaughtering flocks in multiple depopulations was therefore considered a risk factor in the transmission of *Campylobacter* to broilers.

Fomites were washed and disinfected at the processing plant using pressurized chlorinated water. A measurement of the water used indicated that it contained a very high free chlorine content of 72 ppm. Based on sampling consecutive batches of fomites from 05.30 to 12.30 hours over 6 days (Table 2) this study has demonstrated that the proportion of *C. jejuni*-contaminated fomites does not change significantly during the day. Washing thus serves to remove most of the visible dirt while the contamination with viable *C. jejuni* is maintained at a level that could colonize a broiler flock.

This study has identified trucks, forklifts, pallets, crates, drivers' and catchers' boots as potential sources of *C. jejuni* for broilers. These fomites are still contaminated when they depart the processing plant to depopulate broiler flocks despite cleaning. The proportion of contaminated fomites remains the same during the day. Based on the findings of this study, it is recommended that all broiler flocks should be slaughtered in a single depopulation so that the introduction of *C. jejuni* to sheds via fomites would be minimized. It is also recommended that more effective cleaning and disinfection procedures be adopted at processing plants to reduce *C. jejuni* contamination of equipment and worker's footwear prior to visiting poultry farms, and on farms to prevent cross-infection of sheds and other farms.

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