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ORIGINAL ARTICLE

Potential sources of *Campylobacter* infection on chicken farms: contamination and control of broiler-harvesting equipment, vehicles and personnel

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Abstract

Aims: To test the efficacy of enhanced biosecurity measures on poultry farms for reducing environmental contamination with *Campylobacter* during partial depopulation of broiler flocks prior to normal slaughter age. The study has also evaluated the risk of infection from live-bird transport crates that are routinely cleaned at the slaughterhouse, but may remain contaminated.

Methods and Results: On-farm sampling and *Campylobacter* isolation was undertaken to compare the prevalence of contamination on vehicles, equipment and catching personnel during farm visits that took place under normal or enhanced biosecurity. Campylobacters were found in almost all types of sample examined and enhanced biosecurity reduced the prevalence. However, the additional measures failed to prevent colonisation of the flocks. For transport crates, challenge trials involved exposure of broilers to commercially cleaned crates and genotyping of any campylobacters isolated. The birds were rapidly colonised with the same genotypes as those isolated from the cleaned crates.

Conclusions: The enhanced biosecurity measures were insufficient to prevent flock colonisation, and the problem was exacerbated by inadequate cleaning of transport crates at the slaughterhouse.

Significance and Impact of the Study: Current commercial practices in the United Kingdom facilitate the spread of campylobacters among broiler chicken flocks. Prevention of flock infection appears to require more stringent biosecurity than that studied here.

Introduction

Across the European Union (EU), there were 200 507 confirmed cases of campylobacteriosis in 2007 (EFSA 2009). Data from recent case–control studies indicates that the consumption of undercooked poultry meat and handling of raw poultry account for up to 41% of reported human infections with *Campylobacter* spp. (Havelaar *et al.* 2007; Stafford *et al.* 2007; Tam *et al.* 2009). However, source attribution studies using multilocus sequence typing, suggest that poultry is responsible for more than 50% of human cases (Wilson *et al.* 2008; Sheppard *et al.* 2009). During 2008, the prevalence of thermophilic campylobacters in caecal contents of broilers and poultry carcasses at slaughter, determined using standardised protocols, ranged from 2·0–100 and 4·9–100%, respectively, in different EU member states (EFSA 2010). Reducing the number of flocks colonised with *Campylobacter* and subsequently controlling both cross-contamination of carcasses and levels of carcass contamination

at the slaughterhouse is therefore essential in reducing the present incidence of campylobacteriosis in humans (Newell and Fearnley 2003).

Campylobacter is widely distributed in the environment, and it is generally accepted that horizontal transmission of the organism from the environment of the broiler house is the most important route for flock colonisation, which often occurs via human traffic (Newell and Fearnley 2003; Callicott *et al.* 2006). Intervention studies have indicated that good hygienic practices, including well maintained poultry houses with restricted access, and effective use of physical hygiene barriers that involve dedicated footwear and clothing and/or disinfectant footbaths can reduce the risk of transmitting *Campylobacter* to the flock (Berndtson *et al.* 1996; van de Giessen *et al.* 1998; Hald *et al.* 2000). Partial depopulation or 'thinning' of a flock, whereby a proportion of the birds is removed early for slaughter, leaving the remainder to grow to normal clearance age, is practised by a number of large-scale producers in the United Kingdom (Allen *et al.* 2008a). The process, usually undertaken when the flock reaches 30–35 days of age, has been a significant risk factor for flock colonisation in a number of farm-level epidemiological studies (Evans and Sayers 2000; Hald *et al.* 2000, 2001; Adkin *et al.* 2006). The increased risk is associated with the disruption of normal biosecurity practices on the farm and the stress placed on the remaining birds. Following exposure, *Campylobacter* colonisation of these birds develops rapidly, so that high levels of organism may be present in faeces and caecal droppings by the time the flock is cleared, typically 5–8 days following the thinning process (Newell and Fearnley 2003; Allen *et al.* 2008a).

In a previous study of seven farms, we reported an association between *Campylobacter* pulsed-field gel electrophoresis (PFGE) genotypes present on vehicles, transport crates and modules arriving on a broiler farm at thinning and those subsequently recovered from the remaining birds (Allen *et al.* 2008a). Moreover, there was preliminary evidence that particular strains may have spread between farms owned by the same company. Because the thinning crews and associated vehicles often travel from farm-to-farm with their own equipment, boots and working clothes and are likely to have had recent exposure to heavily contaminated environments, a requirement for heightened hygiene control in the thinning process is indicated.

The purpose of the present study, which involved 21 farms managed by a single company, was to determine the effectiveness of additional on-farm biosecurity measures aimed at reducing *Campylobacter* contamination of broiler-harvesting equipment, vehicles and personnel. The study has also taken account of the risk to flock infection from inadequate cleaning of bird transport crates at the slaughterhouse (Tinker *et al.* 2005). Thus, the ability of commercially cleaned crates to cause infection of broilers has been investigated under controlled conditions.

Materials and methods

Farm study plan

In total, 32 separate visits were made to the 21 participating farms over a 1-year period, beginning in March 2007. At these farms, flocks were thinned between 33 and 36 days of age (usually at 35 days) and finally cleared at 41–49 days (usually 46 days). On 16 occasions, biosecurity measures were those being used routinely by the industry at that time (hereafter termed 'normal'). For the other 16 visits, enhanced biosecurity measures were in place during the thinning process. These included the following:

i Cleaning and disinfection of all vehicles entering the farm site. For catching-crew vehicles, pressure washing was used followed by the application of disinfectant over the bonnet and rear of each vehicle and from door-handle level downwards on both sides, including the wheel-arches. In the case of live-bird transporters, the cleaning process covered the wheel-arches, mud-guards and driver steps.

ii Provision of a mobile mess/changing room for the catching crew. This included facilities for hand washing and sanitisation, the use of which was obligatory before staff were allowed to enter the first poultry house and after rest breaks.

iii A requirement for catchers to bring with them fresh clothing, dedicated footwear and any ancillary equipment, including face masks and gloves. Catchers used the changing room on arrival at the farm, and their boots were checked for suitability, cleaned as thoroughly as possible and then disinfected with a 1/120 dilution of Virkon® S (DuPont, Sudbury, UK). The unit was also used for taking refreshments, so there was no need for the catchers to return to their own vehicle, while flock thinning was in progress.

Under normal biosecurity conditions, oncoming vehicles were not cleaned and disinfected at the farm gate. Catchers' hands were not routinely washed, and while footwear was changed, it was not disinfected prior to entering the houses.

Although 11 of the farms were sampled on two occasions (normal and enhanced biosecurity), the remainder were visited only once to fit crop schedules within the timescale of the project. At each farm, samples were collected for microbiological examination, as described later.

Sampling of the birds and farm environment

Birds placed in the first house to be cleared on each farm were designated as the target flock. The house was

Table 1 Sites sampled and types of sample taken at each farm before, during and after flock thinning for all farm visits

Sample no.	Location	Sample type	No. of positive samples/total examined (%)	
			All visits	Flock negative at thinning
1–2	Main driveway and concrete apron	Overshoes	24/64 (37)	4/37 (11)
3–4	Target house	Overshoes	24/60 (40)	1/32 (3)
5	Exterior of catchers' vehicle (wheel-arches, step)	Swab	13/34 (38)	6/20 (30)
6	Interior of catchers' vehicle	Swab	13/32 (41)	5/18 (28)
7	Catchers' hands	Swab	17/93 (18)	6/67 (9)
8	Catchers' footwear	Swab	13/33 (39)	6/19 (32)
9	Exterior of bird transporter	Swab	18/47 (38)	7/24 (29)
9b	Transporter step	Swab	10/18 (56)	6/13 (46)
10	Interior of transporter	Swab	17/46 (37)	6/24 (25)
11	Exterior of forklift	Swab	6/30 (20)	1/16 (6)
12–13	Empty crates	Swab	40/64 (62)	23/36 (64)
14–15	Empty modules	Swab	39/64 (61)	21/36 (58)
16	Exterior of catchers' vehicle after cleaning	Swab	4/10 (40)	2/5 (40)
17	Catchers' hands after cleaning	Swab	6/59 (10)	2/35 (6)
18	Catchers' footwear after cleaning	Swab	4/21 (19)	2/13 (15)
19	Exterior of transporter after cleaning	Swab	5/23 (22)	1/11 (9)
20	Transporter step after cleaning	Swab	1/10 (10)	0/7 (0)
21	Tap water from catching crew unit	Water	0/11 (0)	0/6 (0)
22	Tap before use by catching crew	Swab	1/12 (8)	0/7 (0)
23	Interior of mess unit before catching crew arrive	Swab	0/11 (0)	0/6 (0)
24	Exterior of mess unit before catching crew arrive	Swab	0/11 (0)	0/6 (0)
25	Tap after crew use	Swab	3/11 (27)	1/6 (17)
26	Interior of mess unit after crew use	Swab	5/11 (45)	1/6 (17)
27	Exterior of mess unit after crew use	Swab	4/12 (33)	2/7 (29)
28	Interior of catchers' lunch bags	Swab	3/5 (60)	3/5 (60)
29	Exterior of catchers' lunch bags	Swab	3/5 (60)	3/5 (60)

Samples 1–11, before thinning; samples 12–15, during thinning and before loading; samples 16–20, before thinning, but after cleaning for enhanced flocks only; samples 21–24, additional samples taken before thinning for enhanced flocks only; samples 25–29, additional samples taken after thinning for enhanced flocks only.

sampled by taking 30 faecal droppings on the day of thinning, prior to commencement of the thinning process. For this purpose, the house was divided into six equally spaced sampling areas from which five fresh droppings were collected and pooled. In addition, two overshoe samples were taken from a walk-through of the whole target house. Subsequently, paired caeca were collected from each of 10 carcasses at the processing plant. If *Campylobacter* was not detected at thinning, caeca were again taken from the flock following final clearance. Faecal droppings were also collected from one or more of the adjoining houses on the same farm, depending on the number of houses present.

All sites that were sampled either before, during or after the thinning process, and the type of sample taken in each case are shown in Table 1. The vehicles themselves and the catching personnel were sampled before entering the farm and then again after the enhanced biosecurity measures had been implemented. Each metal-framed module contained 12 open-topped plastic transport crates of the type described by Barker *et al.* (2004).

At each sampling site, an area of *c.* 100 cm^2 was sampled by means of a sterile Readiwipe (Robinson Healthcare Ltd, Chesterfield, UK) premoistened with Maximum Recovery Diluent, MRD (Oxoid, Basingstoke, UK). Several samples from the same site were pooled, e.g. all swabs from the catchers hands and those from the interior and exterior of the live-bird transporter, which included, respectively, the step, wheel-arch and door handle, and the steering wheel, foot pedals and grab handle. When enhanced biosecurity was being practised, swab samples were also taken from the interior and exterior of the mobile unit, both after cleaning but before arrival of the crew and after the crew had used the facility for changing. The main driveway to the farm and the

concrete apron of the target house were sampled using boot swabs, as described by Allen *et al.* (2008a).

All samples were transferred to the laboratory in an insulated cool box and examined within 24 h.

Isolation and identification of *Campylobacter*

Each sample was enriched in modified Exeter broth (Oxoid CM0983, SR0232E, HB034 and Mast Diagnostics supplement SV59) at 37°C for 48 h under microaerobic conditions, prior to subculture onto Oxoid modified charcoal cefoperazone desoxycholate agar (mCCDA), which was incubated microaerobically at 41·5°C for 48 h. Caeca samples were serial diluted and 100 ul of each dilution directly plated onto mCCDA, followed by enrichment as above. The limit of detection for enumeration purposes was taken to be 2×10^2 CFU g^{-1}.

Where possible, three colonies of presumptive *Campylobacter* spp. per sample were subcultured onto Oxoid Blood Agar Base No. 2 (CM 0271) and incubated microaerobically at 41·5°C for 24 h. Confirmation of *Campylobacter* was based on typical cell morphology, production of oxidase, failure to grow in air at 25°C and a positive reaction in the Oxoid Campy Dry Spot Test, as described previously (Allen *et al.* 2008a).

Molecular typing

Isolates from culture-positive samples were genotyped by PFGE using *Sma*I (New England Biolabs, Hitchin, UK), with pulse times increasing from 5 to 40 s and standardised parameters, as proposed by CAMPYNET (http://campynet.vetinst.dk/PFGE.html). Digital gel images of *Sma*I digests were compared using Bionumerics Software (Applied Maths, Kostrijk, Belgium), and cluster analyses were performed with the unweighted pair-group method and arithmetic averages.

To confirm flock and environmental matches, flagellin gene typing of selected strains was undertaken by sequencing of the PCR product of the *fla*A short variable region (SVR) with the primers FLA242FU and FLA625RU (Meinersmann *et al.* 1997). A 321 bp sequence containing the *fla*A SVR nucleotide sequence was then compared with the database at http://pubmlst.org/campylobacter/flaA/ (Dingle *et al.* 2005). Species determination of selected flock and matching environmental isolates was performed by real-time PCR using the method of Best *et al.* (2003).

Genotyping information from 15 flocks was collated and entered together with microbiological data from flock and environmental sampling into a Microsoft Excel® database. Comparative analysis to assess associations between *Campylobacter* genotypes found in the flock and in potential environmental sources was carried out using Fisher's exact test.

Experimental infection of broilers from commercially cleaned transport crates

After approval by the local ethical review committee, two challenge trials were carried out according to the requirements of the Animals Scientific Procedures Act (1986).

In the first trial, three naturally contaminated transport crates were removed after completion of the normal cleaning process at a large UK chicken processing plant. Each crate was plastic wrapped to prevent any further contamination. The crates were transported to the laboratory within 3 h, and on arrival, they were observed to be relatively dry. The interior of the base of each crate was sampled by swabbing, as described by Allen *et al.* (2008a). Clumps of faecal matter that were still attached to the crates were removed, suspended in phosphate-buffered saline and serial tenfold dilutions made in duplicate in the same medium. Samples of drip water from the crates, which had accumulated in the packaging material, were also collected and diluted as described previously. Aliquots (100 μl) were plated on selective agar [Sheep Blood Agar containing Skirrow's supplement, plus actidione and cefoperazone (BASAC)] to obtain viable counts of *Campylobacter* spp. The plates were incubated microaerobically. In case the numbers present were very low, surface swabs and faecal samples were also enriched as described by Allen *et al.* (2008a).

The experimental broilers (Ross) were obtained on day of hatch from a commercial supplier (P D Hook Hatcheries, Bampton, UK). The birds were kept on litter in biosecure accommodation with *ad libitum* access to food and water until they were at least 29 days of age. *Campylobacter*-free status of the birds prior to challenge was established using cloacal swabbing, with samples plated both directly on BASAC agar and after enrichment for 48 h in modified Exeter broth. On the day of challenge, the birds were deprived of food for 3 h and water for 0·5 h, in line with industry practice, before being separated into three equal groups ($n = 20$) in biosecure rooms of 3·5 m × 2·9 m. For groups 1 and 2, a single transport crate was placed in the room with the birds. Food was placed inside each crate to encourage the birds to move in and out freely. The crates were left *in situ* for 24 and 21 h, respectively. A third group of birds was kept in a crate for 3 h in a separate room, but without any food. Subsequently, the crate was transferred to group 2 and placed on top of the crate there to act as a lid. After a 3-h period, all birds were released into the rooms and the crates removed. Ten birds from each

group were killed humanely at 24 h and 3 days postexposure and caecal colonisation levels determined as described previously (Wassenaar *et al.* 1993). Where possible, up to 20 colonies per sample were obtained and subjected to molecular typing.

The second trial was modified from the above, as follows. Four freshly cleaned crates were obtained from the processing plant and sampled as before. Two of the crates were placed in separate rooms and kept there for 45 min to allow contamination of the litter (groups 1 and 2). When the crates were removed, 29-day-old broilers ($n = 20$) were introduced into the rooms, having been kept in biosecure accommodation since the day of hatch. A third group of birds ($n = 20$) was placed in a crate that was housed in a section of a transport module, with an empty crate above to serve as a lid. After 3 h, the birds were removed and transferred to a clean room. Ten birds from groups 1 and 2 were humanely killed for sampling at 2 and 5 days postexposure, and a further 10 from group 3 were killed at 18 h and 5 days postexposure. All were examined for *Campylobacter* as described previously.

Results

Prevalence of *Campylobacter* and levels of flock colonisation

A flock was deemed to be positive if *Campylobacter* was isolated from at least one pooled sample of faecal droppings taken from within the broiler house or from the caeca of slaughtered birds. The flock was considered to be positive at final clearance when at least one of the 10 caecal samples taken at the processing plant was positive. On 14/32 farm visits at the flock-thinning stage, the flock in the target house was already colonised and carried high numbers of *Campylobacter* in the caeca, with a geometric mean of 3×10^6 CFU g^{-1}, although values for individual birds ranged from 2×10^2 to 1×10^{10} CFU g^{-1}. On 16 of the visits, the target flock was negative at thinning, but positive by the time it was cleared at 41–49 days, despite enhanced biosecurity measures on eight of the sites. Levels of caecal carriage ranged from 4×10^4 to 6×10^8 CFU g^{-1}, with a geometric mean of 9×10^7 CFU g^{-1}. On one of the farms, where the target house was negative at thinning and enhanced biosecurity measures were in place, the flock in an adjoining house was found to be colonised. Although the target flock on this farm was positive at clearance, as indicated by positive caecal samples, *Campylobacter* was not recovered from any of the environmental samples. In a further two cases, the target flocks were negative at thinning but, after clearance, caecal samples could not be obtained from the processing plant.

Initial prevalence of contaminated equipment, vehicles and personnel

Table 1 shows that *Campylobacter* was isolated from a high proportion of the sites tested when samples were taken before vehicles and personnel entered the farm. In particular, the catchers' vehicles and transport lorries were often contaminated, despite visual evidence that they had been cleaned prior to arrival. For all vehicles, 41 and 38% were contaminated on the exterior and interior, respectively (Table 1). The former included wheel-arches, door handles and steps, and to elucidate the risk of spread onto the farm, the steps of 18 of the lorries were sampled separately; of these, 10 (56%) were found to be *Campylobacter* positive. The catchers' shoes were also frequently contaminated (39%). As might be expected, the hands of the catching team were contaminated on fewer occasions with 17 of 93 (18%) samples positive but, for each visit, this equated to one or more catchers coming onto the farm with contaminated hands on almost one-third of the occasions.

Crates and modules were not subjected to any additional intervention measures in this study because the necessary cleaning facilities were located at the processing plant rather than the farm. As anticipated, a high proportion of the empty crates (62%), representing 72% of all visits, and modules (61%) from 78% of visits were contaminated. Forklifts were usually brought onto the farm on the day prior to bird harvesting, and of these, 6 of 30 (20%) were found to be positive (Table 1). However, only 1 of 16 samples was positive when the target flock and others were negative. Similarly, the main driveway and concrete apron of the target house were less often contaminated when all flocks were negative (37 and 11% of samples).

Recovery of campylobacters before and after measures on the enhanced biosecurity visits

With regard to the enhanced visits, there was a marked reduction ($P = 0{\cdot}002$) in the prevalence of *Campylobacter* on the catchers' hands and shoes and on the live-bird transporters, after the intensive cleaning procedures (Table 1). The proportion of positive samples from the catchers was reduced by up to 51%, with footwear reduced from 41–19% and hand samples from 14–10%. However, the greatest impact of the enhanced measures was on the transporters, where the proportion of positives from the exterior of the vehicles and the steps was reduced from 53 to 18%. Catchers' vehicles, on the other hand, proved more difficult to clean, and there was little difference in the numbers of positive samples before and after cleaning (Table 1). Despite the observed

improvements, however, the intervention measures had no effect on *Campylobacter* colonisation of the flocks at clearance, because all were positive.

Genotypic associations between isolates from environmental sources at thinning and subsequently recovered from the concomitant flock at clearance

From the 15 visits investigated by molecular typing, 29 genotypes were identified from the 273 isolates recovered from 95 environmental samples. Fifteen genotypes, which were confirmed as *Campylobacter jejuni* and reflected by *fla*A SVR sequence type, were identified from the 79 caecal samples from the concomitant flocks. The diversity of flock genotypes and associated environmental sources are shown in Fig. 1. The number of genotypes identified from each of the 15 flocks at slaughter ranged from 1 ($n = 5$) to 6 ($n = 1$). Approximately, one-third of the environmental samples also yielded more than one genotype with at least one environmental sample on 13 visits matching those from the concomitant flock when slaughtered at clearance (Table 2).

Crates harboured flock-associated strains on seven farm visits and modules on six occasions (Fig. 1; Table 2). Live-bird transport lorry samples also yielded flock-matching strains on seven visits, although only one occasion (T14, genotype PFGE t21/*fla*A SVR 8) was after extra cleaning on the farm. Catchers' vehicles were less likely to yield flock-matching genotypes (three nonenhanced biosecurity visits; Table 2). Despite the high prevalence of campylobacters on catchers' footwear, flock-associated genotypes were identified on only three visits (T5b, T11a and T15) and again neither of these was isolated following on-farm cleaning (Table 2). Likewise, strains matching concomitant flock types were recovered from catchers' hands on two visits (T15, T11a), although only on T11a after washing and sanitisation in accordance with the enhanced biosecurity measures.

Campylobacters recovered from main drive samples matched the subsequent flock colonizing type on two visits and on one of these visits (T15), the strain was indistinguishable (t21/8) to that recovered from a forklift brought onto the farm the previous day.

Interestingly, on Farm T10a, isolates recovered from the interior of a catcher's bag were identified as a match to a genotype (t30/18) later recovered from flock samples (Fig. 1, Table 2).

Overall, 38% of all crate and module samples yielded isolates of genotypes that matched those of the concomitant flock. These sources were more closely associated with flock colonisation than samples from either the thinning crew ($P < 0{\cdot}0001$) or crew vehicles and live transport lorries ($P = 0{\cdot}0131$).

Transport crates as a potential source of flock colonisation

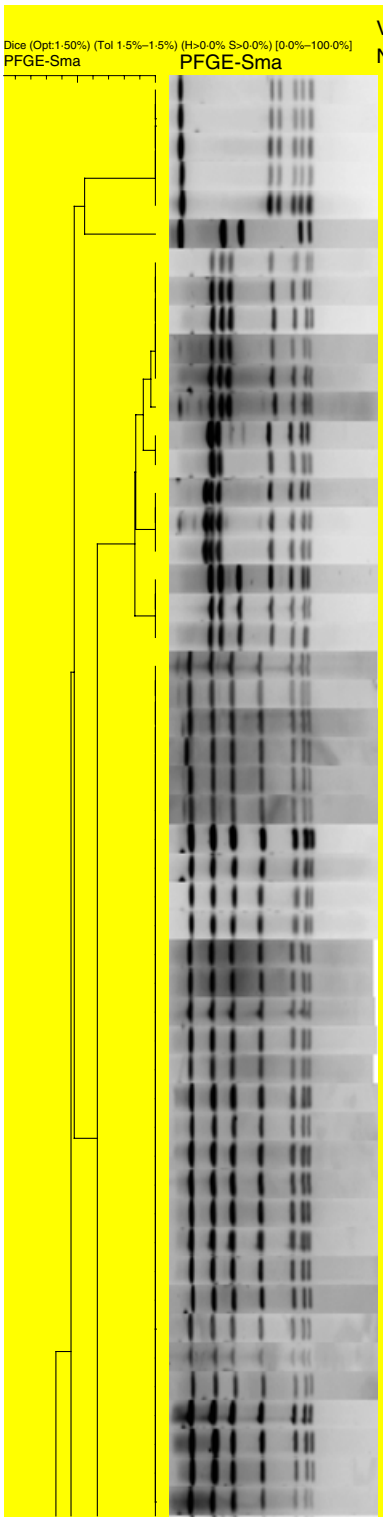
To investigate the infectivity of any campylobacters that remained on the crates after commercial cleaning, broiler chickens were exposed to washed crates under experimental conditions. For the two trials performed, a total of seven crates were brought to the laboratory. Surface swabs of each crate, taken on arrival at the laboratory, were found to be *Campylobacter* positive. Faecal samples from the crates contained 10^6–10^8 CFU g^{-1}, mean 7×10^7 CFU g^{-1}. The residual wash water was also positive (3×10^5 CFU ml^{-1}). Analysis of isolates by PFGE showed that each crate carried at least two distinct strains (range 2–4) in different proportions (Table 3).

In the first trial, using 31-day-old birds, caecal samples taken the day after the start of crate exposure (d1) showed that 6/10 (group 1) and 7/10 (group 2) of the chickens were detectably colonised with *Campylobacter* (Fig. 2). Geometric mean caecal colonisation levels of the colonised birds in these groups were 6×10^4 and 7×10^4 CFU g^{-1}, respectively. Three of the ten birds from the third group were also colonised, despite only having contact with the crate for 3 h (Fig. 2). After 3 days (d3), all birds in each group were fully colonised except one bird in group 3 that had no detectable colonisation (Fig. 2). The genotypes of the strains isolated from the colonised chickens in each group matched those recovered from the corresponding crates (Table 3).

In the second trial, all groups were rapidly colonised. In group 3, 9/10, birds were colonised with *Campylobacter* 18 h after being placed in the module-housed crate and caecal samples contained 10^4–10^8 CFU g^{-1}, with a geometric mean of 2×10^6 CFU g^{-1} (not shown). For groups 1 and 2, caecal samples taken from the birds 2 days following the start of exposure to the crate-contaminated litter yielded 8/10 and 10/10 positives, respectively, with corresponding geometric means of 3×10^4 and 1×10^8 CFU g^{-1}. By 5 days postexposure, the remaining birds in all groups were fully colonised ($>1 \times 10^8$ CFU g^{-1}). PFGE typing showed that two genotypes (t43 and t48), both of which were recovered from all four crates, were the only types recovered from each group of birds sampled at 18 h to 2 days postexposure (data not shown).

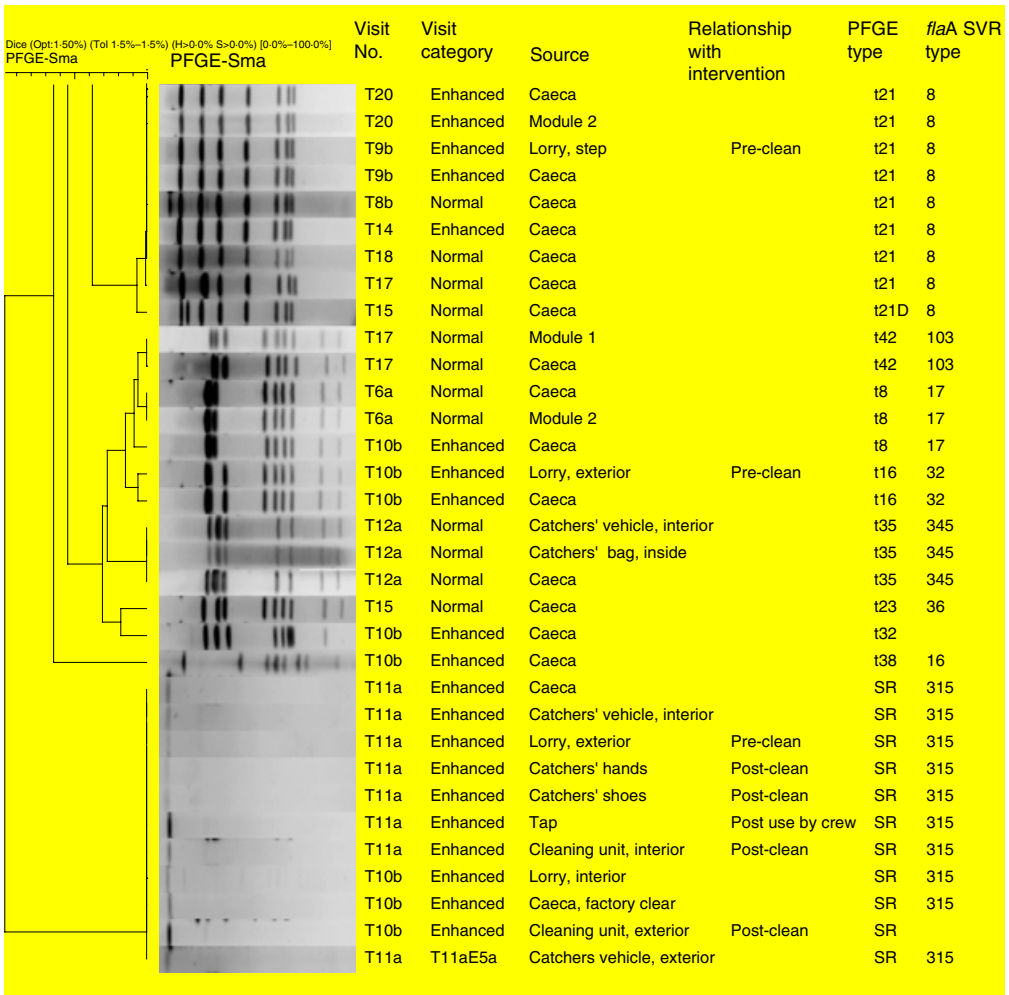
Discussion

These studies have confirmed the observations of Allen *et al.* (2008a) that vehicles, equipment and personnel entering the broiler-farm environment for flock thinning purposes are frequently contaminated with *Campylobacter* and, therefore, present a risk of infection for the remaining



Visit No.	Visit category	Source	Relationship with intervention	PFGE type	*fla*A SVR type
T16	Normal	Main drive		t40	70
T16	Normal	Crate 1		t40	70
T16	Normal	Crate 2		t40	
T16	Normal	Module 1		t40	70
T16	Normal	Caeca		t40	70
T6a	Normal	Caeca		t37	441
T17	Normal	Crate 1		t1A	301
T10a	Normal	Caeca		t1A	301
T20	Enhanced	Caeca		t1A	16
T21	Enhanced	Caeca		t1A	16
T17	Normal	Caeca		t1A	301
T8b	Normal	Caeca		t1A	16
T10b	Enhanced	Caeca		t30A	18
T14	Enhanced	Caeca		t30A	18
T10b	Enhanced	Crate 2		t30	18
T10a	Normal	Caeca		t30	18
T10a	Normal	Catchers' lunch bag, inside		t30	18
T6a	Normal	Caeca		t10	16
T6a	Normal	Module 2		t10	16
T12a	Normal	Caeca		t10A	16
T5b	Enhanced	Caeca		t21	8
T5b	Enhanced	Catchers' shoes	Pre-clean	t21	
T5b	Enhanced	Lorry, exterior	Pre-clean	t21	
T5b	Enhanced	Lorry, exterior	Pre-clean	t21A	8
T5b	Enhanced	Crate 1		t21	8
T5b	Enhanced	Module 1		t21	8
T16	Normal	Caeca		t21	8
T17	Normal	Lorry, step		t21	8
T17	Normal	Crate 1		t21	8
T17	Normal	Crate 2		t21	
T14	Enhanced	Crate 1		t21	8
T14	Enhanced	Crate 2		t21	
T14	Enhanced	Lorry, exterior	Post-clean	t21	8
T14	Enhanced	Catchers' vehicle, interior		t21	8
T20	Enhanced	Lorry, exterior	Pre-clean	t21	
T15	Normal	Crate 1		t21	8
T15	Normal	Catchers' hands		t21	8
T15	Normal	Lorry, interior		t21	
T15	Normal	Forklift		t21	8
T15	Normal	Catchers' hands		t21	
T15	Normal	Catchers' shoes		t21	8
T15	Normal	Caeca		t21	8
T15	Normal	Module 1		t21	
T15	Normal	Main drive		t21	8
T15	Normal	Catchers' hands		t21	8
T9b	Enhanced	Lorry, exterior	Pre-clean	t21	
T18	Normal	Module 1		t21	8
T18	Normal	Module 2		t21	
T17	Normal	Caeca		t21	8
T18	Normal	Crate 2		t21	8

Figure 1 Dendrogram showing *Sma*I pulsed-field gel electrophoresis (PFGE) genotypes recovered from caeca at clear and associated matches to concomitant flock PFGE genotypes identified in environmental samples recovered at the thinning visits of the 15 eligible farms. The band position tolerance was set at 1·5%, and clustering was performed using UPGMA. The scale indicates percentage similarity as determined using the Dice coefficient. Isolate references comprise flock number, category (environment or flock) and sample number. *fla*A short variable region genotypes are also indicated where performed.



Visit No.	Visit category	Source	Relationship with intervention	PFGE type	*fla*A SVR type
T20	Enhanced	Caeca		t21	8
T20	Enhanced	Module 2		t21	8
T9b	Enhanced	Lorry, step	Pre-clean	t21	8
T9b	Enhanced	Caeca		t21	8
T8b	Normal	Caeca		t21	8
T14	Enhanced	Caeca		t21	8
T18	Normal	Caeca		t21	8
T17	Normal	Caeca		t21	8
T15	Normal	Caeca		t21D	8
T17	Normal	Module 1		t42	103
T17	Normal	Caeca		t42	103
T6a	Normal	Caeca		t8	17
T6a	Normal	Module 2		t8	17
T10b	Enhanced	Caeca		t8	17
T10b	Enhanced	Lorry, exterior	Pre-clean	t16	32
T10b	Enhanced	Caeca		t16	32
T12a	Normal	Catchers' vehicle, interior		t35	345
T12a	Normal	Catchers' bag, inside		t35	345
T12a	Normal	Caeca		t35	345
T15	Normal	Caeca		t23	36
T10b	Enhanced	Caeca		t32	
T10b	Enhanced	Caeca		t38	16
T11a	Enhanced	Caeca		SR	315
T11a	Enhanced	Catchers' vehicle, interior		SR	315
T11a	Enhanced	Lorry, exterior	Pre-clean	SR	315
T11a	Enhanced	Catchers' hands	Post-clean	SR	315
T11a	Enhanced	Catchers' shoes	Post-clean	SR	315
T11a	Enhanced	Tap	Post use by crew	SR	315
T11a	Enhanced	Cleaning unit, interior	Post-clean	SR	315
T10b	Enhanced	Lorry, interior		SR	315
T10b	Enhanced	Caeca, factory clear		SR	315
T10b	Enhanced	Cleaning unit, exterior	Post-clean	SR	
T11a	T11aE5a	Catchers vehicle, exterior		SR	315

Figure 1 (*Continued*)

birds. Consequently, new measures to reduce such contamination have been developed and evaluated under commercial conditions. The measures were also designed to prevent or reduce the spread of campylobacters from one farm to another, especially to those with *Campylobacter*-negative flocks. In the present farm study, however, 15 target flocks that were negative at thinning became infected by the time of final clearance, despite the use of enhanced biosecurity measures at some of the farms, both before and during the thinning process. Although the incidence of *Campylobacter* was reduced on vehicles and on the hands and footwear of the catching crew, the organism was still isolated from these sources on many occasions but few of these matched the subsequent flock types, possibly because of a reduction in overall numbers. Footwear, in particular, was difficult to clean effectively, because of the type and/or condition of the boots worn by different individuals. Therefore, there is a need to determine best practice and/or design footwear suitable for bird harvesting that would be easier to clean and disinfect. Another cause of concern was that campylobacters matching the subsequent flock type were even found inside lunch bags on both occasions when these were sampled, indicating the risk of bringing personal items onto the farm.

In addition, there was an apparent problem with the cleaning of transport crates and modules at the slaughterhouse plant, and these were often contaminated with *Campylobacter* on arrival at the farm, as reported previously by Slader *et al.* (2002), Hansson *et al.* (2005) and Rasschaert *et al.* (2007). Standard crate-washing procedures were shown to be largely ineffective in removing campylobacters (Slader *et al.* 2002; Ramabu *et al.* 2004), partly because of difficulties in cleaning the complex

Table 2 Flock colonizing and other genotypes identified in environmental samples from flocks that turned *Campylobacter* positive following thinning

Farm visit*	Visit type	Caecal genotypes† (no. matching isolates/total examined)	Flock-matching environmental sources (genotypes)	Environmental sources not matching flock (PFGE type)
6a	Normal	t10/16 (9/17)	Module	Catchers' vehicle exterior (t7)
		t8/17 (1/17)	Module	
		t37/441 (7/17)	Not detected	
10b	Enhanced	SR‡/315 (7/18)	Lorry interior, unit exterior postclean	Catchers' shoes pre and postclean (t10A/16)
		t38/16 (5/18)	Not detected	Lorry exterior (t4)
		t30A/18 (1/18)	Crates	Modules (t2, t28B)
		t16/32 (2/18)	Lorry exterior	
		t32 (2/18)	Not detected	
		t8/17 (1/18)	Not detected	
10a	Normal	t30/18 (13/14)	Catchers bag, interior	Catchers' bag, exterior (t22)
		t1A/301 (1/14)	Not detected	
11a	Enhanced	SR‡/315 (14/14)	Catchers vehicle interior/exterior, lorry exterior	Catchers' shoes postclean (t29)
			Catchers' hands, catchers' shoes, tap, unit (postclean)	Cleaning unit exterior (t10A)
12a	Normal	t10A/16 (13/15)	Not detected	Catchers' bag exterior, crates, modules (t28)
		t35/345 (2/15)	Catchers' bag interior, catchers' vehicle interior	
14	Enhanced	t21/8 (14/16)	Lorry exterior (postclean), crates, catchers' vehicle interior	None
		t30A/18 (1/16)	Not detected	
15	Normal	t21/8 (10/16)	Main drive, catcher's hands, catcher's footwear, crates, forklift	Catchers' vehicle interior/exterior (t8)
		t23/36 (6/16)	Not detected	Lorry step (t2A)
8b	Normal	t21/8 (13/15)	Not detected	Module, catchers' vehicle exterior (t18)
		t1A/16 (2/15)	Not detected	Module, crate, catchers' hands, catchers' shoes (t2)
9b	Enhanced	t21/8 (11/11)	Lorry exterior	Crate, lorry interior (t38) Catchers' shoes (t30A)
16	Normal	t40/70 (10/14)	Main drive, crates, module	Lorry interior (t28A)
		t21/8 (4/14)	Not detected	
17	Normal	t21/8 (12/14)	Lorry exterior, crates	Module (t30A)
		t1A/301 (1/14)	Crates	
		t42/103 (1/14)	Module	
18	Normal	t21/8 (18/18)	Crates, modules	None
5b	Enhanced	t21/8 (12/12)	Catcher's shoes (preclean), lorry exterior, crate, module	Crate, module
20	Enhanced	t21/8 (14/15)	Modules, lorry exterior	Lorry exterior, module (t17)
		t1A/16 (1/15)	Not detected	Crate (t20)
21	Enhanced	t1A/16 (22/22)	Not detected	Drive, catcher's hands, crates, module, Catcher's hands (postclean), catcher's vehicle (t17)

PFGE, pulsed-field gel electrophoresis.
*Visits are listed in date order.
†The designated type reflects *Sma*I PFGE genotype and *fla*A short variable region sequence type.
‡Confirmed as genotypically related by *Kpn*I PFGE.

plastic surface (Allen *et al.* 2008b). The strains present on washed crates have included genotypes subsequently found in the relevant flocks at clearance (Allen *et al.* 2008a) or following transport (Hansson *et al.* 2007; Lienau *et al.* 2007). Moreover, the colonisation studies presented here have shown that naturally contaminated

Table 3 Pulsed-field gel electrophoresis (PFGE) genotypes of strains recovered from crate samples and exposed chickens during challenge trial 1

Experimental group	PFGE types of strains recovered from crates	PFGE types of strains recovered from exposed birds
1	t44, t45, t47, SR	t44, t45
2	t44, t44a, t46	t44, t44a
3	t1, t45	t1, t45

transport crates can readily infect broilers, with which they come into contact. Handling of contaminated crates by the catchers may cause further dissemination of campylobacters. A large-scale study of risk factors in Iceland (Barrios *et al.* 2006) concluded that catching crews played little or no part in flock infection. However, this may have been because of the use of special hygiene measures and the fact that most of the catchers were farm workers and did not move from one farm to another.

The ease with which broilers were colonised by *Campylobacter* following brief exposure to commercially cleaned crates under experimental conditions was unexpected. Although the birds were not examined until at least 21 h postexposure, which was longer than typical transportation and lairage times in the UK, they were not subjected to the additional stresses associated with commercial transportation that might have increased their susceptibility. However, the findings presented here indicate that birds testing negative at the farm, when the thinning crew arrives, may subsequently carry low, but significant numbers of *Campylobacter* into the processing plant, following transportation. This is in accordance with Hansson *et al.* (2007), who suggested that broilers could be contaminated from the transport crates on the way to slaughter.

Furthermore, broilers became rapidly colonised when placed on fresh litter, which had been in contact with the crates high lighting a potential risk for birds remaining in the house after the thinning process has been completed.

Thinning of flocks is considered a financial necessity by the UK Poultry Industry, a situation that is unlikely to change in the near future. It is therefore vital that further efforts are made to improve the biosecurity of the catching crews, their vehicles and equipment to minimise contamination of the farm environment. Given the difficulties experienced in the present study in reducing such contamination within the logistical and time limitations of on-farm cleaning and disinfection, it may prove more effective to carry out the necessary procedures elsewhere. Any changes in this respect should also aim to improve the cleaning of transport crates at the slaughterhouse.

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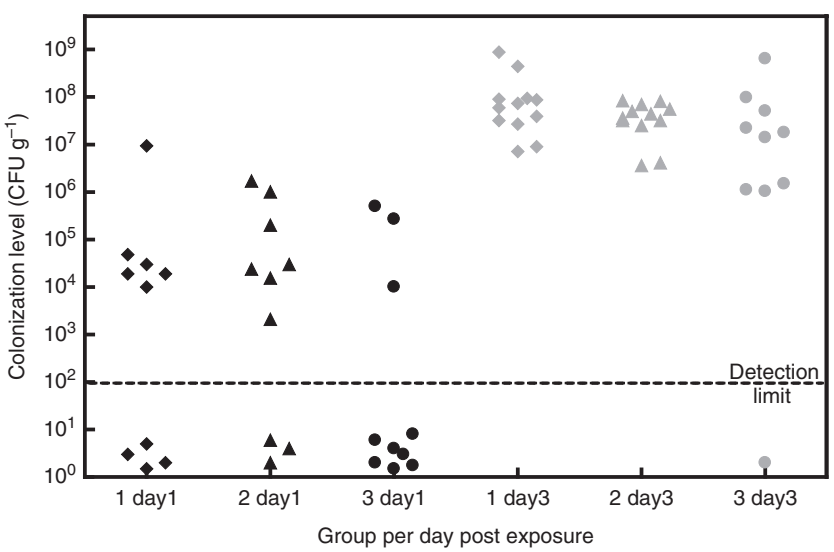

Figure 2 Colonization of 31-day-old broilers at 1 and 3 days postexposure to naturally contaminated washed transport crates.

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