Campylobacter spp. contamination of chicken carcasses during processing in relation to flock colonisation

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Abstract

The presence and numbers of campylobacters on chicken carcasses from 26 slaughter groups, originating from 22 single-house flocks and processed in four UK plants, were studied in relation to the level of flock colonisation determined by examining the caecal contents of at least ten birds per group. The prevalence of campylobacters on carcasses from five campylobacter-negative flocks processed just after other negative flocks was low ($\leq 30\%$). Campylobacters were isolated from 90 to 100% of carcasses from three flocks which were partly colonised, with 5, 5 and 30% of caecal contents positive, and which were processed after fully colonised flocks. All carcasses from the remaining fully colonised flocks were contaminated with campylobacters, and they had significantly ($P < 0.001$) higher numbers per carcass (average of 5.3 $\log_{10}$ cfu; range: 1.3 to >8.0 $\log_{10}$ cfu) than carcasses originating from low prevalence flocks (average of 2.3 $\log_{10}$ cfu; range: <1.1 to 4.1 $\log_{10}$ cfu). There was a reduction in the numbers of campylobacters on carcasses between plucking and chilling in eight of ten fully colonised flocks. In another eight flocks, a significant ($P < 0.001$) decrease (0.8 $\log_{10}$ cfu) in the number of campylobacters on carcasses from just before to after chilling was detected. Campylobacter spp. could be isolated from aerosols, particles and droplets in considerable numbers in the hanging-on, defeathering and evisceration areas but not in the chillers. This was the case even when campylobacters were not isolated from the target flock. Campylobacters on carcasses from two partly colonised flocks were either the same subtype, as determined by speciation, Multi-Locus Sequence Typing (MLST) and flaA Restricted Fragment Length Polymorphism (RFLP) typing, as those in the fully colonised flocks processed previously, although not necessarily the most prevalent ones; or were the same subtypes as those found in the caeca of the flock itself. The prevalences of the different campylobacter subtypes found on carcasses from two fully colonised flocks did not closely reflect those found in the caeca. MLST combined with flaA RFLP provided a good method for ascertaining the relatedness of strains isolated from carcasses and caecal contents. This study showed that carcass contamination is related to the within-flock prevalence of campylobacter colonisation, but that contamination from previously processed flocks was also significant, especially on carcasses from low prevalence flocks. Forced dry air cooling of carcasses reduced contamination levels.

1. Introduction

Campylobacter spp. are thought to be the most common cause of bacterial gastroenteritis worldwide (www.who.int/mediacentre/factsheets/fs255/en). Case–control studies in which the sources of infection were investigated have identified chicken prepared outside the home, handling of chicken and undercooked chicken as important risk factors (Adak et al.,

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2002; Neimann et al., 2003; Friedman et al., 2004; ACMSF, 2005). In some countries it has been found that reductions in the availability of fresh chicken were accompanied by considerable decreases in the incidences of campylobacteriosis (Vellinga and Van Loock, 2002; Stern et al., 2003).

The findings of recent surveys, in a number of countries, of the prevalence and numbers of campylobacters on fresh retail chicken suggest that a considerable proportion of chickens are contaminated, often at a high level (Uyttendaele et al., 1999; Dufrenne et al., 2001; Wilson, 2002; Meldrum et al., 2004). In the UK, a survey which was structured to reflect market share found 56% of chickens on retail sale to be contaminated (Food Standards Agency, 2003) while in another study it was found that 20% of retail chickens carried campylobacters at numbers exceeding 10^5 cfu per carcass (Jørgensen et al., 2002). It has been estimated that there is a considerably increased risk to humans of campylobacter infection associated with high contamination levels in fresh chickens (Rosenqvist et al., 2003).

It may be that the most effective way of reducing the risk to humans posed by contaminated chickens is to reduce the number of flocks colonised with campylobacters. Retailers and the poultry industry, in some countries, have sought to promote this by offering processors and/or farmers incentives relating to the extent to which the chickens they produce are contaminated with campylobacters. However, it has been reported that carcasses sampled towards the end stage of processing have been contaminated with campylobacters even when the bacteria were not isolated from the chickens upon arrival at the abattoir (Berrang et al., 2001; Newell et al., 2001; Slader et al., 2002; Herman et al., 2003; Miwa et al., 2003). In addition, it has been shown that the subtypes of campylobacters found on carcasses from colonised flocks are not always those which were most prevalent in the guts of birds (Newell et al., 2001). It is therefore important to ascertain the extent to which campylobacter colonisation of chickens arriving at the abattoir relates to the degree of contamination of the fully processed carcasses.

Colonisation of a flock by campylobacters can be affected by a practise known as phased depopulation or thinning, where some birds are removed from a house up to two weeks before it is completely depopulated (Hald et al., 2001). However, it is not known whether this affects the level of carcass contamination. There have been many studies of intervention aimed at reducing the degree of cross-contamination of carcasses during slaughter, including heating carcasses' surfaces by steam or hot water, acid sprays, irradiation or the use of chlorinated water (Keener et al., 2004). Previous work has suggested that it is possible to achieve reductions of up to 2 $\log_{10}$ units in numbers of campylobacters on carcasses during processing using chlorinated water (Mead et al., 1995; Stern and Robach, 2003). However, chlorine concentrations higher than those used in potable water are not permitted in the EU, and other studies have found varying effects of using chlorinated water (Mead et al., 1995, 2000; Allen et al., 2000b; Whyte et al., 2001; Li et al., 2002).

The aim of this study was to determine to what extent contamination of carcasses during processing was related to the degree of flock colonisation, ascertained from the proportion of campylobacter-positive caecal contents, and to the thinning of flocks. We also wanted to determine to what extent the campylobacter subtypes found on carcasses were related to the types found in the caeca of the birds in a flock. Changes in the number of campylobacters on carcasses before and after chilling in forced dry air systems, which are the carcass cooling systems most commonly used in the UK, was also determined. Airborne microbial dispersal either as aerosols, water droplets or particulates may also play a major role in cross-contamination during processing (Allen et al., 2000a,b, 2003a,b), so the numbers of airborne campylobacters in the plants at various stages of carcass processing were measured.

2. Material and methods

2.1. Flocks and processing plants

Carcasses from 22 flocks slaughtered in four processing plants in England during 2000 and 2001 were examined. Each flock originated from a single rearing house. None of the flocks, including those preceding the target flocks, were slaughtered first during a day. Carcasses were collected after approximately one third of the birds in a flock had been processed. Each flock was monitored during processing to ensure that targeted birds were sampled. All the plants had line speeds of 100–160 birds min^{-1}, and were similar in design, with an automated processing line passing through a stunner, neck cutter, bleed out, scalding tanks operated at 53±1 °C, a bank of disc plucking machines, eviscerator, vent opener, cropper, neck cracker and puller, and lung remover, followed by air chilling. Plant 1 differed from the other plants in that there was manual rather than automated re-hanging before chilling, onto horizontal rows of shackles ('clip-bars') that moved in sequence through a chilling tunnel with water sprays in the first section. In the other three plants air was directed onto the carcasses as they passed through the chiller on a continuous shackle line, and the chilling times were longer. Some carcasses processed in Plant 3 were subject to a longer chilling process consisting of 8 h in a forced air chiller, followed by 15 h in a maturation chiller operated at about 4 °C. Under normal operating conditions the chilling process consisted of 1 to 1.5 h in a forced air chiller in the other plants.

2.2. Enrichment culture and enumeration of campylobacters

Enrichment culture for *Campylobacter* spp. was based on a method that used a modified Exeter Broth (mEB; Humphrey et al., 1992), which contained Bolton Broth (27.6 g l^{-1}; CM983, Oxoid Ltd., Basingstoke, Hampshire, UK), Campylobacter Growth Supplement (sodium metabisulphate, sodium pyruvate and ferrous sulphate, all at 250 mg l^{-1}; SV61, Mast Diagnostics, Bootle, UK), Campylobacter Selective Supplement (trimethoprim 10 mg l^{-1}, rifampicin 5 mg l^{-1}, polymyxin B 250 IU l^{-1}, cefaperazone 15 mg l^{-1}, amphotericin B 2 mg l^{-1}; Mast, SV59) and lysed defibrinated horse blood (10 ml l^{-1}; E & O Laboratories, Bonnybridge, Scotland). Culture for enrichment was performed in containers with <1 cm of head space and tightly closed lids, at 37 °C for 48 h. After enrichment, 10 µl of the enrichment broth was

streaked onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA; Oxoid, CM739 with SR155 supplement) and the plates were incubated at 37 °C for 48 h in gas jars with a microaerobic atmosphere (Bolton et al., 1992). Microaerobic atmospheres were created by evacuating two-thirds of the volume of air in each jar and refilling it with a gas mixture of 10% CO_2, 10% H_2 and 80% N_2. This gave an atmosphere of 5–6% O_2, 3–7% CO_2 and 7% H_2, with the balance being nitrogen.

Presumptive campylobacter colonies were confirmed by a positive oxidase reaction, microscopic appearance following Gram staining and lack of growth in air at 25 °C. In addition, a latex agglutination kit (Campylobacter Test Kit; Oxoid, DR 0150 M) was used to confirm the identities of approximately 50% of the isolates. These strains were retained for later typing and speciation.

Numbers of campylobacters in samples were determined by plating undiluted and/or 10-fold dilutions of each sample prepared using Maximum Recovery Diluent (MRD; Oxoid, CM733) onto mCCDA and incubating the plates as described above. Presumptive colonies were counted, and 3 to 6 colonies per plate were confirmed as described above.

2.3. Sampling and examination of caecal contents

Pairs of caeca were collected after evisceration and the contents (~ 1.5 g) of one caecum were removed through an incision at the blind end. For flocks F, G, H, I, J, L, N, R, S, T, U and V the surfaces of intact caeca were sterilised by swabbing with 70% industrial methylated spirit and flaming before removal of the contents. A sample of the contents of each caecum was examined by direct streaking of ~ 0.03 g onto mCCDA, enrichment of 1 g in 9 ml of mEB and plating appropriate ten-fold dilutions in MRD onto mCCDA. The numbers of presumptive campylobacters were expressed as $\log_{10}$ cfu g^{-1} caecal contents.

2.4. Sampling of broiler carcasses

Fresh disposable gloves were worn to remove each carcass from the processing line. Each carcass was placed in a sterile plastic bag, and carcasses were transported to the laboratory in insulated boxes with ice packs. From each carcass, 25 g of neck skin was removed and placed in a stomacher bag. Three hundred millilitres of sterile deionised water was poured through the vent of the carcass into the abdominal cavity, and the carcass was rinsed for 1 min by shaking the bag 30 times in each of two directions to ensure that the water came into contact with all chicken surfaces. The rinse fluid was then poured into the stomacher bag containing the neck skin and the bag was stomached for 1 min, using a 'Lab-Blender 400' stomacher (Seward, London, UK). Campylobacters were enumerated by plating 500 µl of ten-fold dilutions of stomached fluid in MRD onto each of two mCCDA plates. For enrichment culture, 25 ml of stomacher fluid was transferred to a sterile plastic container, and 225 ml of mEB was added.

2.5. Examination of airborne contamination

Air was sampled in four areas of each processing plant, at hanging-on, defeathering, evisceration and chilling of carcasses,

during the processing of flocks A, B, C, D, E, F, G, H and M (Tables 1–4). Sampling of airborne contamination including droplets and particulate matter was performed using sedimentation (settle) plates. Plates of mCCDA and Colombia blood agar (COLBA; Oxoid, CM331supplemented with 5% defibrinated horse blood from E & O Laboratories) were exposed to the air for either 1 or 5 min. They were incubated as described above for mCCDA plates, and the numbers were expressed as the mean deposition rate (cfu cm^{-2} min^{-1}). The detection limit was 0.02 cfu cm^{-2} min^{-1}.

The contents of each COLBA plate was placed in a sterile plastic container with 225 ml of mEB and enriched as described above.

Sampling of aerosols and particulate matter 10 µm or less in size was performed using Burkard samplers (Burkard Manufacturing Co Ltd., Rickmansworth, UK). The samplers were operated for 5 min at a flow rate of 20 l min^{-1}. The agar plates used were as above, but they contained 37 ml agar per plate. The numbers of presumptive *Campylobacter* spp. were expressed as cfu l^{-1} of air and the detection limit was 0.05 cfu l^{-1}. Air was monitored during processing of flocks A and B, flocks F and G and flocks C, D, E, H and M using settle plates, Burkard samplers, or both plates and samplers, respectively.

2.6. Sampling and examination of scald tank water

Scald tank water (25 ml) was collected by immersing sterile plastic containers in it. The water was transported to the laboratory, on ice in an insulated container or portable refrigerator, for enrichment culture and enumeration of campylobacters as described above.

2.7. Subtyping of campylobacters

Speciation using real time PCR (Best et al., 2003), serotyping by the heat-stable antigen scheme of Frost et al. (1998), and phage typing (Frost et al., 1999) was performed at

Table 1
Incidence of campylobacter contamination of carcasses from campylobacter-negative flocks

Plant	Target flock	No. of positive samples/no. examined		
		Preceding flock, caecal contents[a]	Target flock, caecal contents[a]	Target flock, chilled carcasses[b]
1	C	0/10	0/10	0/5
2	C	0/10	0/10	0/5
1	D	0/10	0/10	0/5
2	D	0/10	0/10	0/5
1	E	0/10	0/10	0/5
2	E	0/10	0/10	0/5
3	K	1/14	0/28	2/30[c]
3	L	0/59	0/73	9/30[d]

[a] Caecal contents were examined by direct plating and enrichment culture.
[b] Detection limit=1.1 $\log_{10}$ campylobacters per carcass.
[c] One carcass sample also examined by enumeration contained 2.5 $\log_{10}$ cfu per carcass.
[d] Two carcass samples also examined by enumeration each contained<2.5 $\log_{10}$ cfu per carcass.

Table 2
Campylobacters on carcasses from flocks with a low prevalence of colonisation

Plant	Target flock	No. of positive caecal contents/no. examined[a]		No. of carcasses positive by enumeration/no. examined (mean $\log_{10}$ CFU per carcass, SE)[b, c]		
		Preceding flock	Target flock	After Plucking	After evisceration	After chilling
3	I	14/14	2/59	2/5 (2.1, 0.5)	18/30 (2.5, 0.2)	14/30 (2.4, 0.1)
3	F	10/10	3/10	ND	ND	3/5 (3.4, 0.4)
3	N	10/10	1/20	ND	ND	5/5 (3.6, 0.2)

ND: not done.

[a] Caecal contents from Flocks I and N were positive by enrichment culture only. One caecum from Flock F was positive by direct plating while the other two were positive by enrichment only.

[b] Campylobacters were detected by enrichment (detection limit = $\log_{10}$ 1.1) in all carcasses in which they were not detected by enumeration (detection limit 2.5 $\log_{10}$ cfu) except from three Flock I post chill carcasses.

[c] The mean was calculated by assigning a value of 1.8 $\log_{10}$ cfu for carcasses positive by enrichment but not by direct plating and a value of 0.6 $\log_{10}$ cfu for the three carcasses which were negative by enrichment.

the *Campylobacter* Reference Unit (CRU), Health Protection Agency, Colindale, London. In the serotyping scheme, strains were labelled according to which antiserum gave the strongest reaction, as many isolates cross reacted with several antisera. Some strains were also characterized using Multi Locus Sequence Typing (MLST; Dingle et al., 2001) and *flaA* Restricted Fragment Length Polymorphism (RLFP) as some strains were untypable using sero- and/or phage typing. FlaA RLFP typing was performed using the protocol recommended by the CAMPYNET research forum (http://campynet.vetinst.

Table 3
Mean $\log_{10}$ numbers of campylobacters on carcasses from flocks with high prevalences of infection

Plant	Flock	Mean $\log_{10}$ cfu g^{-1} caecal contents (SE)[a]	Mean $\log_{10}$ cfu per carcass (SE), $n = 5$		
			After plucking	After evisceration	After chilling
4	A (1)[b]	ND[c]	4.2 (0.1)	3.8 (0.3)	3.8 (0.5)
4	A (2)[b]	9.1 (0.4)	5.0 (0.3)	5.1 (0.5)	4.1 (0.3)
4	A (3)[b]	8.7 (0.5)	7.3 (0.3)	6.5 (0.4)	6.2 (0.2)
4	B (1)[b]	ND[c]	6.8 (0.5)	5.4 (0.1)	4.3 (0.1)
4	B (2)[b]	8.7 (0.3)	6.8 (0.2)	5.9 (0.1)	6.3 (0.2)
4	B (3)[b]	9.6 (0.2)	7.8 (0.3)	6.4 (0.2)	6.7 (0.7)
1	M	4.4 (0.9)	5.0 (0.1)	5.9 (0.5)	5.7 (0.2)
3	G	6.3 (0.4)	5.3 (0.4)	4.6 (0.1)	2.6 (0.7)
3	H	7.9 (0.2)	5.5 (0.4)	6.1 (0.4)	5.5 (0.2)
3	J	6.6 (0.6)	6.9 (0.4)	6.2 (0.3)	6.2 (0.4)

[a] Campylobacters were enumerated in 10 to 14 caecal contents per flock. All were positive by enumeration except for two from Flock M, which were only positive by enrichment culture. A value of 1.8 $\log_{10}$ cfu g^{-1} was assigned to samples positive by enrichment but not direct plating.

[b] Phased depopulation at (1) 37 or (2) 44 days, or (3) clearance at 51 days.

[c] Caecal contents not examined at the first depopulation but an average (SE) of 5.4 (0.3) and 5.1 (0.2) $\log_{10}$ cfu campylobacters g^{-1} were isolated from fresh faecal samples collected from the chickens on the day of slaughter for Flocks A and B, respectively.

Table 4
Mean $\log_{10}$ cfu of campylobacters on chicken carcasses from high prevalence flocks before and after chilling

Plant	Flock	Chilling process[a]	Mean $\log_{10}$ cfu per carcass before chilling (SE, n)[b]	Mean $\log_{10}$ cfu per carcass after chilling (SE, n)[b]
4	O	S	5.9 (0.1, 20)	5.8 (0.1, 20)
4	P	S	5.4 (0.2, 20)	4.7 (0.2, 20)
4	Q	S	6.5 (0.1, 20)	6.0 (0.1, 20)
3	R	L	6.4 (0.1, 30)	5.8 (0.1, 30)
3	S	L	5.9 (0.2, 15)	4.9 (0.2, 15)
3	T	L	5.5 (0.1, 15)	4.4 (0.1, 15)
3	U	L	7.0 (0.3, 15)	5.0 (0.2, 15)
3	V	L	5.7 (0.2, 15)	5.1 (0.3, 15)
		S		4.7 (0.3, 15)

[a] The shorter chilling process (S) normally operated under in Plants 3 and 4. The longer chilling process (L) operated in Plant 3 as part of an experimental chilling process trial.

[b] Samples collected before chilling were obtained after the final carcass wash, except with Flock R for which they were obtained after evisceration. SE = standard error of the mean; n = the number of carcasses examined for each pre- and post-chilling value.

dk/Fla.htm), with amplification of the full *flaA* gene and digestion using *Dde*I. The protocol was modified so that digestion was performed using 1 μl of sterile ultrapure water, 2 μl of 10× enzyme buffer, 1 μl of 5 U μl^{-1} *Dde*I (New England Life Sciences, Hitchin, UK) and 16 μl of flagellin PCR product. Other reaction conditions were as published.

2.8. Analysis of results

Analysis of differences in the campylobacter prevalence on chill carcasses from different flocks was performed using a chi-squared test. Analysis of variance for sets of log transformed counts was performed using Minitab software, to determine whether the numbers of campylobacters on carcasses or in caeca differed between flocks. Analysis of variance for a bird within-flock and flock within plant hierarchy was performed using MlwinN v2, (Institute of Education, London, UK), to test whether the numbers of campylobacters on carcasses differed when the effects of chilling were investigated in more detail.

3. Results

Arbitrary limits were set to define the campylobacter status of the flocks studied. Thus, flocks were referred to as negative when campylobacters were not detected in <10 caeca, low prevalence when ≤30% of the caeca were campylobacter-positive or high prevalence when >30% of caeca were positive.

3.1. Levels of campylobacters on carcasses in relation to the prevalence and numbers in caeca at slaughter

Campylobacters were detected on 30% of carcasses in one of four negative flocks which were slaughtered after other negative flocks and in 7% in one negative flock slaughtered after a flock colonised at a low prevalence (Table 1). Very low numbers of

Table 5
Relationship between *Campylobacter* spp. subtypes detected in caecal contents and on carcasses

Sample type (n)/source	Species (*n*, n)[a]	MLST sequence type (*n*, n)	Phage/serotype (*n*)[b]	*flaA* (*n*)[c]
Caeca (10)/ Flock before Flock F	*C. coli* (28, 10)	1680 (*3*, 1)	UT/56 (*2*), /24 (*1*)	F5 (*3*)
		1681 (*8*, 8)	1/56 (*4*), /24 (*1*); 44/56 (*2*), /48 (*1*)	F13 (*8*)
		–	1/56 (*7*), /48 (*4*); 44/56 (*4*), /48 (*2*)	F13 (*17*)
Caeca (2)/ Flock F	*C. coli* (12, 2)	860 (*10*, 2)	44/56 (*7*), /UT (*3*)	F12 (*10*)
		887 (*1*)	44/24 (*1*)	F15 (*1*)
		–	1/48 (*1*)	–
Carcasses I (5)/Flock F[d]	*C. coli* (22, 4)	–	7/56 (*6*), /UT (*2*); 2/UT (*1*); UT/UT (*1*)	F16 (*10*)
		–	44/56 (*5*)	F18 (*5*)
		–	44/24 (*2*); 1/24 (*1*); UT/24 (*1*)	F5 (*4*)
		–	44/48	F10 (*1*)
		–	44/56 (*1*)	–
	C. jejuni (12, 3)	–	25/5 (*3*), /UT (*3*), /23 (1)	–
		–	33/UT (*3*); 14/50 (1); 30/UT (1)	–
Carcasses II (5)/Flock F[e]	*C. coli* (24, 4)	1680 (*12*, 4)	1/24 (*9*); 44/24 (*1*); UT/24 (*1*), /56 (*1*)	F5 (*12*)
		–	1/24 (*8*); 44/24 (*3*); UT/24 (*1*)	F5 (*12*)
	C. jejuni (7, 2)	–	25/5 (*6*)	F6 (*6*)
		–	29/UT (*1*)	F5 (*1*)
Caeca (14)/Flock before Flock I	*C. coli* (13, 13)	–	–	F9 (*9*)
		–	–	F10 (*3*)
		–	–	F1 (*1*)
	C. jejuni (1, 1)	53	–	F1 (*1*)
Caeca (2) Flock I	*C. jejuni* (5, 2)	53 (*4*, 2)	–	F1 (*5*)
Carcasses II (24)/Flock I	*C. coli* (10, 10)	–	–	F9 (*4*)
		–	–	F10 (*5*)
		–	–	F12 (*1*)
	C. jejuni (14, 14)	53 (*11*, 11)	–	F1 (*11*)
		573 (*1*)	–	F1 (*1*)
		50 (*1*)	–	F7 (*1*)
		606 (*1*)	–	–
Caeca (5)/Flock before Flock G	*C. jejuni* (12, 5)	–	2/18 (*12*)	–
Caeca (10)/Flock G	*C. jejuni* (26, 10)	45 (*6*, 4)	67/27 (*5*), /45 (1)	F1 (*6*)
		45 (*17*, 9)	67/27 (*5*), /13 (2), /45 (*3*); UT/13 (2), /45 (*5*)	–
		354 (*3*, 2)	2/18 (*3*)	F2 (*2*)
Carcasses I/(5) Flock G	*C. jejuni* (13, 5)	45 (*4*, 3)	UT/UT (*3*), /45 (*1*)	F1 (*1*)
		354 (*3*, 3)	2/18 (*1*), –	F2 (*2*)
		–	2/18 (*3*); UT/45 (*1*), /27 (*1*), /UT (*1*)	–
Carcasses II (10)/Flock G	*C. jejuni* (23, 10)	45 (*1*)	UT/45	F1
		354 (*4*, 4)	2/18 (*3*)	F2 (*1*)
		–	2/18 (*18*)	F2 (*1*)
Caeca (11)/Flock before Flock H	*C. jejuni* (24, 11)	48 (*2*, 2)	–	–
Caeca (14)/Flock H	*C. coli* (28, 12)	–	2/39 (*4*); 44/UT (*1*)	F5 (*5*)
	C. jejuni (14, 9)	791 (*12*, 7)	–	–
		354 (*2*, 2)	–	–
Carcasses II (10)/Flock H	*C. coli* (28, 10)	–	–	F5 (*6*)
	C. jejuni (1)	791 (*1*)	–	–

[a] (*n*, n): (number of strains, number of samples).

[b] When /serotype is preceded by a comma, isolates have the same phage type as the previous isolate; UT = untypable or reacted with phage but did not conform to a recognised type.

[c] *fla*A restriction fragment length polymorphism type. Mean band size measured manually (standard error), F1: 172 (4), 231 (2), 256 (1), 329 (2), 610 (4); F2: 152 (3), 231 (1), 939 (7); F5: 161 (1), 224 (2), 281 (3), 896 (4); F6: 159, 187, 223, 271, 410; F7: 162, 190, 229, 302, 333; F9: 140, 165, 207, 264, 749; F10: 170, 197, 239, 270, 290; F12: 169, 237, 269, 294, 441; F13: 219, 1116; F15: 163, 236, 316, 345, 404; F16: 168, 195, 228, 264, 306; F18:171, 223, 265, 744.

[d] Carcasses I were removed just after the wash performed after evisceration.

[e] Carcasses II were removed after chilling.

campylobacters were detected on some of these carcasses. Significantly ($P<0.05$) more carcasses from the low prevalence flocks were contaminated with campylobacters as compared to carcasses from negative flocks, and the former carcasses were also contaminated with higher numbers of campylobacters (Table 2). All carcasses from the high prevalence flocks were contaminated with campylobacters, and after chilling they carried significantly ($P<0.001$) higher numbers of campylo-

bacters (Table 3) than carcasses originating from the low prevalence flocks (Table 2). For Flocks A and B, with which phased depopulation was practised, significantly ($P<0.001$) fewer campylobacters were detected on chill carcasses from birds removed during the first depopulation than on carcasses from birds removed during the final clearance (Table 3). However, no correlation was found overall between the numbers of campylobacters detected in caeca and on carcasses.

3.2. Numbers of campylobacters on carcasses in relation to processing

With most flocks, the numbers of campylobacters per carcass were less after eviscerating and after chilling than after plucking (Table 3). To determine to what extent numbers of campylobacters on carcasses were affected by the chilling process, carcasses from eight fully colonised flocks were examined just before and after chilling (Table 4). There were no significant differences ($P > 0.05$) between carcasses subjected to a longer or shorter chilling process, or between plants. Analysis of the combined data showed that, overall, numbers were significantly ($P < 0.001$) lower by 0.8 $\log_{10}$ cfu (SD=0.8) on chilled carcasses than on carcasses removed before chilling.

3.3. Presence of campylobacters in the air of processing plants

No campylobacters were isolated from any of the air samples collected from the chiller areas of processing plants, regardless of flock status. Campylobacters at 2 and 110 cfu 0.01 m^{-2} min^{-1} were detected in air samples collected using settle plates during the processing of the campylobacter-negative Flock E in the hanging-on, plucking and evisceration areas of the processing plant. Of the eight air samples collected with the Burkard sampler one was positive, yielding 5 campylobacters 100 l^{-1}. No campylobacters were isolated from any air sample collected during the processing of two other negative Flocks, C and D. Campylobacters were detected in air using the Burkard sampler in the evisceration area only during the processing of the low prevalence Flock F, while none of the air samples collected during the processing of Flock M were positive. During the processing of flocks A, B and H with high campylobacter prevalence, campylobacters at 2 to 50, 10 to >300 and 9 to >300 cfu 0.01 m^{-2} min^{-1} were detected in the hanging-on, plucking and evisceration areas of the processing plants, respectively. Campylobacters at 1 to 32 cfu 100 l^{-1} were detected during the processing of Flock G.

In the scald tank water, 3.9 and 3.1 $\log_{10}$ cfu campylobacters ml^{-1} were found while Flocks A and B, respectively, were being processed.

3.4. Campylobacter spp. on carcasses in relation to those found in the caeca

The campylobacters found on carcasses from Flock F, which was a low prevalence flock, were mainly *C. coli*, but *C. jejuni* were also present. Most of the *C. coli* strains from the chilled carcasses were of a subtype (ST1680, F5) indistinguishable from that of some isolates from the high prevalence flock slaughtered before Flock F. In addition, several strains (*C. jejuni* and *C. coli* F16 and F18) detected on the carcasses of Flock F after the wash were not detected either in the caeca from the preceding flock or in the caeca from Flock F itself (Table 5). The phage/serotyping results suggested that some of the *C. coli* subtypes found on the carcasses from Flock F were identical to those found in the caeca of the flock itself, but they were different MLST and flaA RFLP types. About a third of the strains recovered from chilled carcasses from Flock I, a low prevalence flock, were indistinguishable by

flaA RFLP typing (*C. coli* F9 and F10) from the most common strains found in the high prevalence flock slaughtered before it, while another common subtype (*C. jejuni* ST53) was the same as one found in the caeca of birds from Flock I itself.

The most common subtype (ST354, PT2HS18) on the chilled carcasses from Flock G was also detected in the caeca of birds in the flock. The fraction of this subtype in the caeca was, however, much smaller than the fraction of the subtype in campylobacters on the carcasses. Nearly all campylobacters isolated from the carcasses of Flock H were *C. coli*, and a majority of the strains isolated from the caecal contents of the birds were the same *C. coli* subtype (F5). In contrast, only one of 29 isolates from carcasses was of the same *C. jejuni* subtype (ST791) as that found in 9 of the caeca from 14 birds in Flock H. None of the *C. jejuni* strains from carcasses were of the subtype (ST48) detected in the fully colonised flock processed before Flock H.

4. Discussion

We detected cross-contamination on carcasses from two of five campylobacter-negative flocks, even when these were processed after other negative ones. In other studies in UK plants, undertaken in 1997–1998 (Newell et al., 2001; Slader et al., 2002), prevalences of cross-contaminated flocks of 3/3 and 1/2 were found, while in studies in Belgium (Herman et al., 2003) and Japan (Miwa et al., 2003) 1/6 and 4/7, respectively, were found. We detected between 16 and 30% while others have reported between 47 and 100% positive carcasses in cross-contaminated flocks (Herman et al., 2003; Miwa et al., 2003). Such prevalences probably reflect the status of preceding flocks, differences between processing plants and the sampling/detection methods used. For example, flocks where no cross-contamination of carcasses was detected were almost all either processed first in the day or preceded by negative flocks only, as in this study. Plant 3 in our study routinely processed extensively-reared birds, i.e. birds which are allowed outside the growing sheds during rearing, first in the day. Such birds have been shown to be frequently contaminated with campylobacters (Heuer et al., 2001). No extensively-reared birds were processed in Plants 1 or 2. Although the prevalence of cross-contamination can be high, the numbers of campylobacters on the carcasses are likely to be relatively low, ranging from 1.1 (Slader et al., 2002) to the 2.5 $\log_{10}$ cfu per carcass found in this study. Contamination of carcasses during processing can occur at various points, including during scalding and plucking.

Campylobacters were isolated from nearly all carcasses from the low prevalence flocks examined in this study. In the Belgian study (Herman et al., 2003), however, only 1/30 and 11/60 carcasses from two partly colonised flocks were positive. As well as reflecting the status of preceding flocks this could also be related to the method of examination and type of carcass sample examined, which differed between the two studies. Recent studies have found that the type of sample examined and the enrichment method used can affect isolation rates of *Campylobacter* spp. from chicken carcasses (Jørgensen et al., 2002; Corry et al., 2003; Paulsen et al., 2005). The low prevalence of campylobacters in some flocks could reflect their very recent exposure to campylobacters, perhaps just before slaughter.

While there was no notable difference in the prevalence of campylobacters on carcasses from the high and low prevalence flocks, there was a significant difference in the numbers. This finding agrees with studies on carcasses processed in one US plant (Stern and Robach, 2003). It is likely that such a difference would significantly affect the risk of contracting campylobacteriosis from chicken (Rosenquist et al., 2003). It was not possible, however, to establish a direct relationship between the numbers of campylobacters in caeca from the high prevalence flocks and the extent to which carcasses originating from these flocks became contaminated. A similar finding was reported by Stern and Robach (2003), who were unable to relate numbers of campylobacters in faeces to the levels detected on carcasses.

The within-flock variation in the numbers of campylobacters on carcasses reported in this paper has also been observed by Stern and Robach (2003), and they suggested this could relate to differences in colonisation levels in individual birds within a flock. However, it could also relate to whether the carcass examined was subject to a variable level of contamination due to visceral breakage or leakage during evisceration, or reflect inconsistent removal of campylobacters from carcasses during sampling. Berrang et al. (2001) demonstrated that contamination with only 5 mg of caecal material caused a significant increase in the numbers of campylobacter on a carcass. High numbers of campylobacters can be recovered after repeated rinses of the same carcass (Jørgensen et al., 2002). We also found differences between high prevalence flocks in the levels of campylobacters on carcasses examined after chilling. This may relate to the campylobacter status of flocks processed previously, or to the bacteria having different sensitivities to the stresses encountered during processing, and/or to the antibiotics in the mCCDA plates used to enumerate them.

The contamination of carcasses was usually lower after chilling than after plucking. In one plant, however, with manual re-hanging and water sprays in the first section of the chiller, and a short process time, there was no such reduction. These findings are in agreement with previous studies (Allen et al., 2000a) in which similar results for total microbial numbers on carcasses were found with this type of chiller. Although chilling without water sprays was found to reduce the number of campylobacters on carcasses, the reductions were relatively small and variable. Previous studies by Oosterom et al. (1983) gave similar results. These authors suggested that drying caused by forced ventilation rather than cooling is the main factor responsible for the reduction of numbers of campylobacters on poultry and pig carcasses. However, drying may be less effective during air chilling of poultry than of pig carcasses because of the shorter cooling times for poultry. In addition, the shape of the carcass and skin texture may also protect the campylobacters on chickens from dehydration. Evidence to support this has been reported by Hudson and Roberts (1982) who isolated C. jejuni from moist but not dry areas of pig carcasses. This suggests that greater reductions could occur with increased drying of poultry carcasses.

Campylobacters were isolated in considerable numbers from aerosols, particles and droplets in the hanging-on, plucking and evisceration areas of the processing plants but not in the chillers.

This occurred even when the organism was not isolated from either the target flock or the one preceding it. Oosterom et al. (1983) and Berndtson et al. (1996) also isolated campylobacters from air in the same three areas of other plants, but in the later study campylobacters were also recovered from air around the spin chiller. The use of water sprays in air chillers may enhance microbial dispersal (Allen et al., 2000a).

The campylobacters on carcasses from two partly colonised flocks were the same subtype as on the fully colonised flocks processed previously, or were either the same subtypes as those found in the caeca of the flock itself or were subtypes not detected in either caeca from the flock itself or the previously processed one. The campylobacters on carcasses from fully colonised flocks were mainly the same subtypes as found in the caeca of the flocks themselves although, in one flock, strains from the flock processed before the target flock may also have contributed to the contamination of carcasses. The contamination of carcasses from one fully colonised flock did not reflect closely the prevalence of the subtypes found in the caeca of this flock, and this could possibly be due to differential survival of strains during processing.

MLST provided an unambiguous and universal method for assessing the relatedness of strains while sero-/phage typing occasionally failed to provide a type, and sometimes did not provide unambiguous results.

Acknowledgements

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