Evaluation of Different Plate Media for Direct Cultivation of *Campylobacter* Species from Live Broilers

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ABSTRACT Accurate identification and optimal culturing procedures for *Campylobacter* spp. from live broilers are needed for epidemiological studies. Because there is no standardized protocol, we designed and conducted studies to evaluate different selective media for the culturing and isolation of *Campylobacter* spp. from cecal and fecal samples obtained from battery-reared and commercial broilers. Five media selective for *Campylobacter* were evaluated: *Campylobacter* agar base, *Campylobacter*, Campy-Line, modified Campy-Cefex, and modified charcoal cefoperazone deoxycholate agar. With contaminated broilers reared in battery cages, *Campylobacter* agar base, *Campylobacter*, modified Campy-Cefex, and modified charcoal cefoperazone deoxycholate agar revealed similar isolation rates ($P > 0.05$), whereas Campy-Line showed a lower efficacy ($P < 0.05$). With commercial live broilers, modified Campy-Cefex agar was more consistent for the isolation of *Campylobacter* from feces, whereas modified Campy-Cefex and modified charcoal cefoperazone deoxycholate agar showed similar isolation rates from cecal samples. Campy-Line agar showed a lower identification rate ($P < 0.05$) for both fecal and cecal samples. A multiplex PCR assay used for identification showed that *Campylobacter jejuni* and *Campylobacter coli* DNA was present in the samples. Pulsed field gel electrophoresis restriction profiles differed among samples collected from different commercial farms but were similar for isolates from the same farm, suggesting clonal differences. No variation was seen in pulsed field gel electrophoresis patterns among isolates cultured on different media. Our data suggest that the choice of plate medium may influence the efficiency of isolating *Campylobacter* spp. from broiler chickens by direct plating from fecal or cecal samples.

Key words: *Campylobacter*, broiler, plate media

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INTRODUCTION

Campylobacter spp. are commonly found in the intestines of avian species as a natural host (Luechtefeld et al., 1980; Yogasundram et al., 1989; Oyarzabal et al., 1995; Wallace et al., 1998; Newell and Fearnley, 2003), including market-aged commercial broilers (Oosterom et al., 1983; Wempe et al., 1983; Newell and Fearnley, 2003). The principal sites of colonization of the bacteria in chickens, with the highest incidence and numbers, are the ceca, large intestine, and cloaca, where *Campylobacter jejuni* cells pervade the lumina of crypts without attaching to crypt microvilli (Beery et al., 1988). Experimentally, as low as 40 viable colony-forming units of *C. jejuni* can colonize chickens (Cawthraw et al., 1996). However, the colonization patterns in chickens depend on the *C. jejuni* strain (Ringoir and Korolik, 2002), but it has been estimated that in a flock of about 20,000 broilers, the prevalence of *Campylobacter* would increase from 5 to 95% within 6 d after the introduction of viable *Campylobacter* cells (van Gerwe et al., 2005), and exposure to *Campylobacter*-contaminated water results in the colonization of the birds in the flock within 7 d (Shanker et al., 1990). The intestinal carriage of campylobacters appears to be a major contamination factor for broiler carcasses (Oosterom et al., 1983), influences the final microbial quality of the carcass after processing (Musgrove et al., 1997), and is a potential source of human campylobacteriosis (Grant et al., 1980).

Several cultivation media have been used for the isolation of campylobacters from human feces (Skirrow, 1977; Lauwers et al., 1978; Butzler and Skirrow, 1979; Bolton and Robertson, 1982; Butzler et al., 1983; Goossens et al., 1983, 1986; Bolton et al. 1984). For the isolation of campylobacters from the intestinal tract of chickens, enrichment of the samples has been commonly used in inoculation experiments (Dhillon et al., 2006) or in naturally

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occurring colonization studies (Jones et al., 1991; Humphrey et al., 1993; Pearson et al., 1993; Shreeve et al., 2000). However, the use of direct plating of fecal samples may be a faster method for isolation of *Campylobacter* from fecal samples (Shanker et al., 1990; Kazwala et al., 1992; Kapperud et al., 1993; Jacobs-Reitsma et al., 1994). Charcoal cefoperazone deoxycholate agar is commonly used worldwide (Bolton et al., 1984; Hutchinson and Bolton, 1984; Kazwala et al., 1992; Jacobs-Reitsma et al., 1994), although Preston agar (Bolton and Robertson, 1982; Kapperud et al., 1993), Campy-Cefex agar (Stern et al., 1992), and variations of published media containing different concentrations of antimicrobials have also been used (Chattopadhyay et al., 2001). The selectivity in these media is given by different antimicrobials, but difficulties in interpretation appear when contaminants grow.

The goal of our study was to evaluate different cultivation media for isolation of *Campylobacter* spp. from fecal samples collected from broilers. In our first experiment, fecal material was collected from broilers inoculated with a *C. jejuni* strain. In the second experiment, we evaluated 3 agar plates for the isolation of naturally occurring *Campylobacter* spp. from fecal and cecal samples collected from commercial broilers.

MATERIALS AND METHODS

Sample Collection

Experiment 1. Sixty broiler chickens were placed in battery cages (2 pens per cage). Birds were housed in 3 batteries, designated groups A through C (replicates). A standard feed treatment was given to the birds at placement. Birds were challenged with a strain of *C. jejuni* in the drinking water (~10^3 cfu of *C. jejuni*/mL). This strain of *C. jejuni* (mCC-248) has been isolated in our laboratory from retail broiler samples (Oyarzabal et al., 2005). The challenge with the *C. jejuni* strain started 2 h after placement and lasted for 48 h. Three birds per group were euthanized with CO_2 on d 8, 21, 28, and 35, and their ceca were removed, weighed, and serially diluted in PBS. Dilutions were plated onto *Campylobacter* agar base (BD Laboratories, Franklin Lakes, NJ), *Campylobacter* agar plates (**CAP**; Oyarzabal et al., 2005), modified charcoal cefoperazone deoxycholate agar (**mCCDA**; Hutchinson and Bolton, 1984), Campy-Line agar plates (**CL**; Line, 2001), and modified Campy-Cefex agar plates (**mCC**; Oyarzabal et al., 2005). Table 1 shows the composition of each of the agar plates. Plates were incubated at 42°C for 48 h under microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2; Airgas, Radnor, PA) using anaerobic jars with a MACSmics jar gassing system (Microbiology International, Frederick, MD). Suspect colonies were examined, counted, and confirmed using phase-contrast microscopy. Presumptive *Campylobacter* isolates from all countable plates were collected and stored at −80°C in tryptic soy broth (Difco, Detroit, MI) supplemented with 30% glycerol (vol/vol) and 5% blood for further confirmation and subtyping.

On d 4 and 15, three fecal samples from each group of birds were collected to determine the colonization status. Serial dilutions were made in PBS and plated on mCC. After 48 h of incubation under microaerophilic conditions, plates were counted and presumptive colonies confirmed for typical *Campylobacter* shape using phase-contrast microscopy.

Experiment 2. Fecal samples were collected from commercial broiler farms for the analysis of *Campylobacter* spp. Samples were collected on 6 independent trips, in which 3 houses from 3 different farms were sampled per trip (total = 18 houses representing 18 farms). Ten 1-g fecal samples were weighed per house and immediately added to 9 mL of Preston broth. Samples were kept under microaerophilic conditions generated with CampyGen (Oxoid Ltd., New York, NY) and transported (~4°C) to the laboratory for analysis.

Cecal samples were also collected in 2 trips. Five birds per house were euthanized, and their ceca were removed, weighed, and placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI). Preston broth was added to obtain a ratio of 1:9 (wt/vol). Samples were kept under microaerophilic conditions as described above. Each bird was considered a replicate for statistical purposes.

At the laboratory, fecal samples were pooled in 2 groups of 3 samples each and 1 group of 4 samples (3 replicates) for *Campylobacter* analysis. Both fecal and cecal samples were serially diluted in PBS and plated in duplicates on mCC, mCCDA, and CL. Plates were incubated at 42°C for 48 h under microaerophilic conditions. Samples were also enriched in Preston broth for 24 h under microaerophilic conditions at 42°C and then transferred to mCC for *Campylobacter* detection. All presumptive isolates from the countable plates were collected and stored at −80°C in tryptic soy broth (Difco) supplemented with 30% glycerol (vol/vol) and 5% blood. These isolates were identified with a multiplex PCR assay and characterized by pulsed-field gel electrophoresis (**PFGE**).

Campylobacter Identification

Unambiguous identification of the bacteria was done with a rapid hippurate test kit (Hardy Diagnostics, Santa Maria, CA) and a specific multiplex PCR assay for the identification of *C. jejuni* and *Campylobacter coli* (Oyarzabal et al., 2007). Briefly, bacterial DNA was extracted using PrepMan Ultra (Applied Biosystems, Foster City, CA) and tested with a PCR assay that targets the aspartokinase gene specific for *C. coli* (Linton et al., 1997), the hippuricase gene specific for *C. jejuni*, and the 16S ribosomal DNA gene that is specific to the genus (Persson and Olsen, 2005). Polymerase chain reaction assays were performed in 25-μL aliquots in a PTC-100 programmable thermal controller (BioRad, Hercules, CA). Amplicons were detected by standard gel electrophoresis in 1.5% agarose and DNA bands stained with ethidium bromide and visualized using a ultraviolet transilluminator (Gel-Doc system, Syngene, Frederick, MD) with a molecular analyst computer program (Syngene).

Table 1. Composition of the media used in the experiments (per-liter basis)

Medium	Base	Supplements[1]
CAB[2]	Proteose peptone (15 g/L)	Amphotericin B (2 mg/L)
	Liver digest (2.5 g/L)	Cephalothin (15 mg/L)
	Yeast extract (5 g/L)	Polymyxin bisulfite (250 units/L)
	Sodium chloride (5 g/L)	Sheep blood (100 mL/L)
	Agar (12 g/L)	Trimethoprim (5 mg/L)
	Deionized water (1 L)	Vancomycin (10 mg/L)
		Amphotericin B (2 mg/L)
CAP[3]	*Brucella* agar (43 g/L)	Lysed horse blood (70 mL/L)
	Ferrous sulfate (25 mg/L)	Novobiocin (5 mg/L)
	Sodium bisulfite (25 mg/L)	Polymyxin B (250 units/L)
	Sodium pyruvate (25 mg/L)	Trimethoprim (5 mg/L)
	Deionized water (1 L)	Vancomycin (10 mg/L)
CL[4]	*Brucella* agar (43 g/L)	Hemin (10 mg)
	Ferrous sulfate (0.5 g/L)	Polymyxin B sulfate (0.35 mg)
	Sodium bisulfite (0.2 g/L)	Trimethoprim (5 mg)
	Sodium pyruvate (0.5 g/L)	Vancomycin (10 mg)
	α-Ketoglutaric acid (1 g/L)	Cycloheximide (100 mg)
	Sodium carbonate (0.6 g/L)	Cefoperazone (33 mg)
	Deionized water (1 L)	Triphenyltetrazolium chloride (200 mg)
mCC[5]	*Brucella* agar (43 g/L)	
	Ferrous sulfate (0.5 g/L)	
	Sodium bisulfite (0.2 g/L)	Laked horse blood (50 mL)
	Sodium pyruvate (0.5 g/L)	Cefoperazone (33 mg)
	Deionized water (1 L)	Cycloheximide (0.2 g)
mCCDA[6]	Nutrient broth no. 2 (25 g/L)	
	Bacteriological charcoal (4 g/L)	
	Casein hydrolysate (3 g/L)	
	Sodium desoxycholate (1 g/L)	
	Ferrous sulfate (0.25 g/L)	
	Sodium pyruvate (0.25 g/L)	
	Agar (12 g/L)	Cefoperazone (32 mg)
	Deionized water (1 L)	Amphotericin B (10 mg)

[1]All supplements were purchased from Sigma-Aldrich (St. Louis, MO) except the horse blood that was obtained from the School of Veterinary Medicine, Auburn University, Alabama (Institutional Animal Care Committee Protocol PRN 2004-0623).

[2]Purchased from BD Laboratories (Franklin Lakes, NJ), BBL *Campylobacter* agar. CAB = *Campylobacter* agar base.

[3]Cephalothin replaced by cefoperazone and sheep blood by lysed horse blood.CAP = *Campylobacter* agar plates.

[4]CL = Campy-Line agar plates.

[5]mCC = modified Campy-Cefex agar plates.

[6]*Campylobacter* selective blood free agar (CM0739) purchased from Oxoid Ltd. (New York, NY). Cefoperazone and amphotericin B purchased from Sigma-Aldrich. mCCDA = modified charcoal cefoperazone deoxycholate agar.

PFGE Analysis of Isolates

All *Campylobacter* isolates were subtyped using PFGE by comparing the patterns of *Sma*I restriction endonuclease digests. We followed previously described protocols (Ribot et al., 2001; CDC, 2006) and used a contour-clamped homogeneous electric field (BioRad) with 1% agarose gels that were stained with ethidium bromide and visualized with a ultraviolet transilluminator (Gel-Doc system, Syngene). Pair comparison and cluster analysis was done using the Dice correlation coefficient and the unweighted pair group mathematical average clustering algorithm of BioNumerics version 4.50 (Applied Maths, Austin, TX). The optimization and position tolerance for band analysis were set at 1%. A cutoff of 90% was used for the determination of the different PFGE patterns (De Boer et al., 2000).

Statistical Analyses

Bacterial counts were converted to base-10 logarithm colony-forming units per gram of fecal and cecal material. Means were analyzed for differences using Duncan's test (GLM procedure of SAS), and the SE was calculated with PROC MEANS (SAS Institute Inc., Cary, NC). For all tests, a $P \leq 0.05$ was considered significant.

RESULTS

The results from cecal samples of experiment 1 are summarized in Table 2. We detected a lower *Campylobacter* count (average 6.8 $\log_{10}$ cfu/g) in birds at 8 d of age than in birds at 21 d (7.8 $\log_{10}$ cfu/g) and 35 d (8.1 $\log_{10}$ cfu/g). *Campylobacter* agar base, CAP, mCC, and mCCDA had a similar performance ($P > 0.05$) and resulted in the highest counts in all sampling days, except for d

Table 2. Recovery of *Campylobacter* spp. ($\log_{10}$ cfu/g) from cecal samples on different plate media by sampling days (experiment 1)

Day	Plate[1]	Cecal content	SE
8	CAB	7.1^{AB}	0.33
	CAP	6.8^{AB}	0.27
	mCCDA	7.5^{A}	0.44
	CL	6.1^{B}	0.67
	mCC	7.2^{AB}	0.25
21	CAB	7.9^{AB}	0.15
	CAP	7.6^{AB}	0.21
	mCCDA	8.2^{A}	0.17
	CL	7.2^{C}	0.21
	mCC	8.3^{A}	0.17
28	CAB	8.3^{A}	0.14
	CAP	8.1^{AB}	0.17
	mCCDA	8.4^{A}	0.16
	CL	7.6^{B}	0.19
	mCC	8.3^{A}	0.21
35	CAB	$8.5^{A,B}$	0.15
	CAP	8.2^{B}	0.19
	mCCDA	8.7^{A}	0.13
	CL	6.5^{C}	0.24
	mCC	8.3^{B}	0.11

$^{A-C}$Means within a column lacking a common superscript differ ($P < 0.05$).

[1]CAB = *Campylobacter* agar base; CAP = *Campylobacter* agar plates; mCCDA = modified charcoal cefoperazone deoxycholate agar; CL = Campy-Line agar plates; mCC = modified Campy-Cefex agar plates.

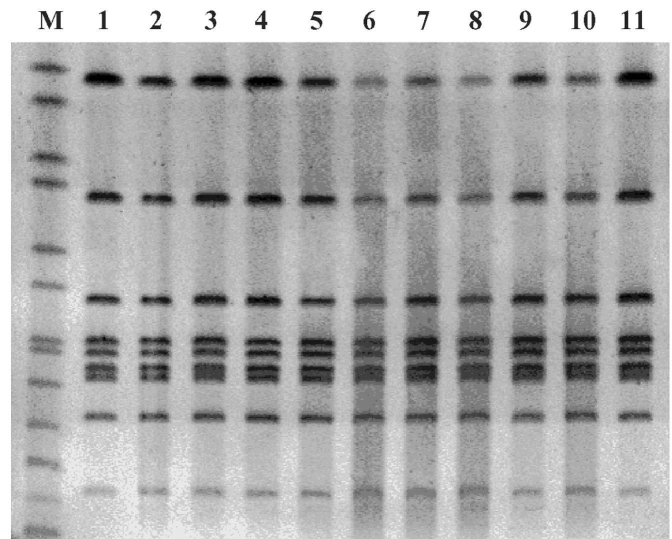

Figure 1. Restriction profiles of selected isolates from experiment 1. M = *Salmonella choleraesuis* ssp. *choleraesuis* serotype Braenderup H9812 (ATCC BAA-664) marker. 1 = *Campylobacter jejuni* control; 2 through 6 = *C. jejuni* isolates collected on d 8, 21, 28, and 35; 7 through 11 = isolates collected on d 35 (modified charcoal cefoperazone deoxycholate agar: 7, modified Campy-Cefex agar plates: 8, *Campylobacter* agar base: 9, *Campylobacter* agar plates: 10, Campy-Line agar plates: 11).

35, in which CAP and mCC had a lower count ($P < 0.05$) compared with mCCDA (Table 2). Campy-Line agar plates exhibited the lowest counts when compared with the other media ($P < 0.05$) in all sampling days, especially on d 35 when CL had 1.7 $\log_{10}$ cfu of *Campylobacter* per gram of cecal material lower than the second lowest medium (CAP). Fecal counts of *Campylobacter* spp. from each treatment group collected on d 4 and 15 showed colonization values that varied from 7.5 to 8.0 $\log_{10}$ cfu of *Campylobacter* spp. per gram of fecal material, respectively. Figure 1 shows that the restriction profiles of the *C. jejuni* strain inoculated in the chickens and the restriction profiles of the isolates collected on d 8, 21, 28, and 35 were the same. In addition, isolates collected from the same bird on different media on d 35 revealed the same restriction profile patterns. The restriction profile of the inoculated strain did not vary throughout the study.

The results from the analyses of fecal samples from experiment 2 are shown in Table 3. Ten farms (total = 18) were positive for *Campylobacter* by fecal analysis, whereas 3 farms (total = 6) were positive for *Campylobacter* by cecal analysis. All farms positive by cecal analysis were also positive by fecal analysis. Positive flocks had a large variation ($P < 0.05$) in the number of colony-forming units of *Campylobacter* spp. that were isolated per flock by fecal analysis (Table 4). By cecal analysis, farms had 2.7 up to 4.3 $\log_{10}$ cfu of *Campylobacter* spp. per gram. No variation ($P > 0.05$) was observed in the *Campylobacter* counts from the same farm by cecal or fecal analysis (results not shown). Of the 3 media compared for the recovery of naturally occurring *Campylobacter* from broiler samples, mCC showed the best results, followed by mCCDA. As with the results from experiment 1, CL exhibited the lowest counts ($P < 0.05$).

Thirty-nine isolates were kept for hippurate testing, PCR, and PFGE analysis. Of these isolates, 36 (92%) were *C. jejuni* and the rest (8%) were a mix of *C. jejuni* and *C. coli*. The PFGE analysis showed that there was a predominant isolate for each farm, although the isolates from different farms had unique restriction patterns (Figure 2). However, in a few samples, more than 1 *C. jejuni* strain was isolated from the same farm with a low percentage of relatedness between them (Figure 3). No correlation was observed between the restriction pattern of the isolates and any particular plate medium.

DISCUSSION

Because *Campylobacter* spp. are a major cause of gastroenteritis, there is a need to control these pathogens in live broiler chickens to reduce the counts in the final food products. Although several media designed for the isolation of *Campylobacter* from fecal samples in humans or food samples have been used to test live broilers, we do not know if there is any variation in the performance of these media. This information becomes more relevant if studies are performed to determine the reduction in numbers of *Campylobacter* spp. by any given intervention. The results from inoculation studies showed that plates with either blood or charcoal had a better recovery rate than CL. In a study comparing the efficacy of CL and Campy-Cefex in different samples taken from 18 carcasses, Campy-Cefex performed better ($P < 0.05$) than CL to culture *Campylobacter* spp. from cecum and colon samples. These cecum and colon samples also had the

Table 3. Recovery of *Campylobacter* spp. ($\log_{10}$ cfu/g) from broiler fecal and cecal samples from commercial farms on different plate media (experiment 2)

	Feces		Cecal content	
Plate[1]	Mean	SE	Mean	SE
mCC	5.7^{A}	0.21	4.5^{A}	0.20
mCCDA	4.1^{B}	0.33	4.1^{A}	0.45
CL	2.4^{C}	0.35	1.2^{B}	0.10

$^{A-C}$Means within a column lacking a common superscript differ ($P < 0.05$).

[1]mCC = modified Campy-Cefex agar plates; mCCDA = modified charcoal cefoperazone deoxycholate agar; CL = Campy-Line agar plates.

lowest correlation coefficients (0.87 and 0.88, respectively) between the 2 media (Line and Berrang, 2005).

Data from the evaluation of media to isolate *Campylobacter* spp. from naturally infected birds corroborated these results. It is not clear whether the lack of blood or charcoal, that are thought to be oxygen-quenching compounds that improve the microaerophilic conditions for the growth of *Campylobacter* spp. (Corry et al., 1995); the concentration of antimicrobials, as suggested for the lower counts found in carcass rinses (Oyarzabal et al., 2005); or a combination of both factors account for the lower enumeration rate obtained with CL. The antimicrobials in media used for the detection of *Campylobacter* may inhibit the growth of some strains of *C. coli* and *C. jejuni* (Ng et al., 1985). In a study of the effects of various antibiotics on a variety of *Campylobacter* spp., Loades et al. (2005) found that all commercial *Campylobacter* selective media tested were inhibitory to some strains of *C. jejuni* and *C. coli*. In addition, antibiotic-sensitive *Campylobacter* strains have been isolated from human feces by the use of membrane filtration onto antibiotic-free agar (Steele and McDermott, 1984; Lastovica, 2006).

All media supplemented with either blood or charcoal—regardless of the number of antimicrobials or the presence of oxygen-quenching supplements—resulted in statistically similar counts for *C. jejuni*. It appears that the oxygen-quenching properties of blood and charcoal may play a role on the direct isolation of *C. jejuni* from fecal or cecal samples. They also allowed for the isolation of *C. coli* strains, which were not found in CL. Research has shown that the use of cefoperazone in charcoal-based media did not appear to hinder the isolation of *C. coli* from swine feces (Gun-Munro et al., 1987).

The collection of samples from commercial broiler flocks showed that 44% of the flocks were negative for *Campylobacter* by fecal and cecal analyses and that the positive flocks had a large variation in the number of colony-forming units of *Campylobacter* spp. that were isolated per flock. The sampling of the commercial broiler farms was performed in the summer (2005 and 2006), when the prevalence of *Campylobacter* spp. is the highest. The number of negative flocks is lower than a survey carried out in 2001, in which 87% of commercial broiler flocks (total = 32 flocks) were *Campylobacter* positive (Stern et al., 2001). It is apparent that if the flock is positive, the number of *Campylobacter* will be countable in plates, because enrichment does not result in an increase of positive samples. Similarly, direct plating for isolation of *Campylobacter* spp. from human feces has been used for

Table 4. Enumeration of *Campylobacter* spp. ($\log_{10}$ cfu/g) by commercial farm from broiler fecal samples (experiment 2)

Farm number	Mean[1]	SE
1	1.8^{C}	0.42
3	3.3^{BC}	0.65
5	4.1^{B}	0.81
6	6.9^{A}	0.22
7	5.3^{AB}	0.17
8	4.1^{B}	0.81
9	3.5^{BC}	0.65
10	4.1^{B}	0.83
12	3.5^{BC}	0.49
13	4.2^{B}	0.61

$^{A-C}$Means within a column lacking a common superscript differ ($P < 0.05$).

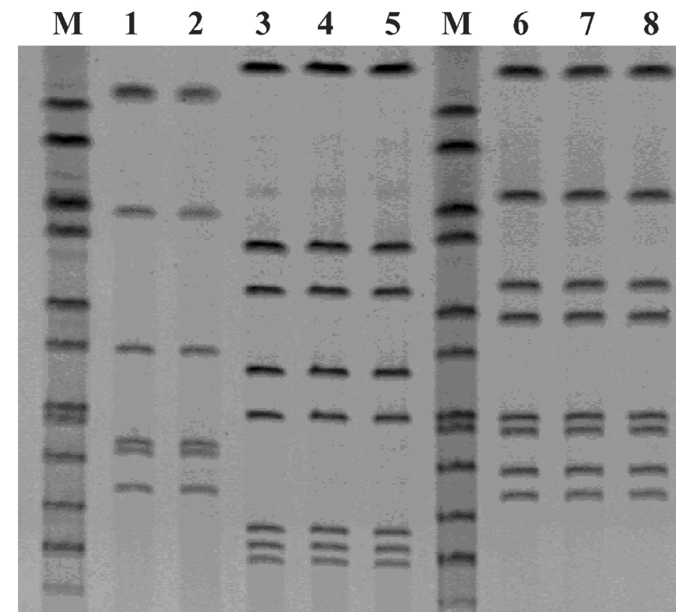

Figure 2. Restriction profiles of *Campylobacter jejuni* isolates collected from 3 farms on different sampling days (experiment 2). M = *Salmonella choleraesuis* ssp. *choleraesuis* marker. 1 and 2 = samples from farm 3, second sampling day; 3 through 5 = samples from farm 2, fourth sampling day; 6 through 8 = samples from farm 3, forth sampling day; 1, 3, and 6 = isolates from modified Campy-Cefex agar plates; 2, 4, and 7 = isolates from modified charcoal cefoperazone deoxycholate agar plates; 5 and 8 = isolates from Campy-Line agar plates.

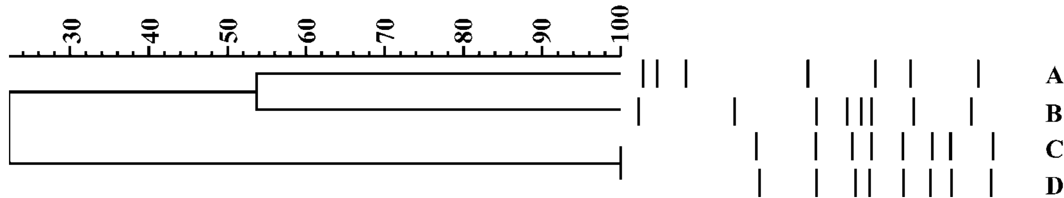

Figure 3. *Campylobacter jejuni* isolates collected from fecal samples from 2 farms on the same sampling day. C and D = isolates from farm 1; A and B = isolates from farm 2; A and C = isolates from modified Campy-Cefex agar plates; B and D = isolates from modified charcoal cefoperazone deoxycholate agar plates. The DNA percentage relatedness was calculated using the UMPGA analysis by BioNumerics (Applied Maths, Austin, TX).

more than 2 decades, because the enrichment steps do not result in an increase in the number of positive samples (Bolton et al., 1984). An efficient technique for the isolation of a variety of *Campylobacter* spp. from human feces has been developed (Lastovica, 2006). This technique uses filtration through a 0.6-μm pore-size filter onto antibiotic-free isolation plates. Combined with incubation in a H_2-enriched microaerophilic atmosphere, *Campylobacter* isolations from pediatric stools increase from 7.1 to 21.8% (Lastovica, 2006). It would be of interest to do a direct comparison of this technique and antibiotic-containing agar isolation plates for the detection of *Campylobacter* strains from broiler feces.

In a previous study in which swab samples were taken from adult laying hens, an improvement of 40% in positive samples was achieved when duplicate samples were transported in semisolid motility test medium (Chan and MacKenzie, 1982), with 1 sample cultured directly and the other enriched first in the semisolid medium (Sjögren et al., 1987). In the case of *Campylobacter* isolation from a cattle slaughter facility, both direct plating and selective enrichment were proposed as the optimal combination for surveillance of *C. jejuni* in fecal material from cattle (Gharst et al., 2006).

In experiment 2, the 3 kinds of agar plates still allowed for the growth of some contaminants. The majority of these contaminating bacteria presented different colony morphology and were easily differentiated from *Campylobacter* colonies. This was verified by the use of phase-contrast microscopy, which was invaluable to presumptively identify *Campylobacter* colonies from contaminants. A predominant strain appears to colonize the chickens in a farm, but the presence of mix cultures of *C. jejuni* and *C. coli* suggest that the presence of more than 1 *C. jejuni* strain may be common and underestimated. The low percentage of DNA relatedness among the strains from the same farm suggests that horizontal entry is an important source of contamination for broiler chickens. A combination of 2 or more *C. jejuni* strains carried by a commercial flock has been demonstrated (Thomas et al., 1997), although it may be unnoticed if no fingerprinting assays are performed. Unfortunately, these assays are not regularly performed on *Campylobacter* strains isolated from broiler feces. The coexistence of different isolates and even 2 species has been detected by direct plating and in enriched samples (Oyarzabal et al., 2007). Therefore, when collecting all the growth from a plate, chances are that more than 1 strain or more than 1 species of *Campylobacter* will be saved. It is important then to highlight the challenges of obtaining single, isolated colonies on agar plates for PFGE analysis from *C. jejuni* and *C. coli* (Barrett et al., 2006). In these cases, the drying of the agar plates and the use of filtration membranes are useful for identification of single colonies on plates.

Direct plating can be used successfully for isolation of *Campylobacter* from broiler samples. The medium chosen may affect the recovery of *Campylobacter* spp. Considering performance and cost (Oyarzabal et al., 2005), mCC and mCCDA appear to be the media of choice for isolation of *Campylobacter* from fecal and cecal samples from broiler chickens. In addition, sampling of commercial flocks on d 35 may allow for the analysis of the samples closer to market age, which would in turn be the most appropriate time to identify if the flock will be positive or not for *Campylobacter* before processing. In the future, this information may be of value to the industry if a logistic scheduling process is incorporated to reduce the chances of contamination of free flock during processing.

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