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Prevalence of *Campylobacter* spp. in Cattle in Finland and Antimicrobial Susceptibilities of Bovine *Campylobacter jejuni* Strains▽

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The study investigated the prevalence of *Campylobacter* spp. in Finnish cattle at slaughter and carcass contamination after slaughter. During the period January to December 2003, bovine rectal fecal samples (n = 952) and carcass surface samples (n = 948) from 12 out of 15 Finnish slaughterhouses were examined. In total, campylobacters were detected in 31.1% of fecal samples and in 3.5% of carcass surface samples. *Campylobacter jejuni* was isolated from 19.5%, *Campylobacter coli* from 2.2%, and presumptive *Campylobacter hyointestinalis* from 10.8% of fecal samples. Campylobacters were detected in 4.4% and 37.4% of the fecal samples examined both by direct culture and by enrichment (n = 730), respectively, suggesting a low level of campylobacters in the intestinal content. A slightly increasing trend was observed in the overall prevalence of campylobacters towards the end of summer and autumn. Seventeen different serotypes were detected among the fecal *C. jejuni* isolates using a set of 25 commercial antisera for serotyping heat-stable antigens (Penner) of *C. jejuni* by passive hemagglutination. The predominant serotypes, Pen2 and Pen4-complex, were isolated from 52% of the fecal samples. Subtyping by pulsed-field gel electrophoresis (SmaI) yielded 56 and 20 subtypes out of 330 fecal and 70 carcass *C. jejuni* isolates, respectively. MICs of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline for 187 *C. jejuni* isolates were determined using a commercial broth microdilution method. Sixteen (9%) of the isolates were resistant to at least one of the antimicrobials tested. Resistance to nalidixic acid was most commonly detected (6%). No multiresistance was observed.

Over the last 20 years thermophilic campylobacters have become the most important human bacterial pathogens in most western European countries (55a). In Finland the number of reported cases has shown an increasing trend over the last 10 years apart from a slight decrease from 2002 to 2003 (35). In Finland, during the seasonal peak from June to September in 2003 approximately 40% of the cases were of Finnish origin (53).

Poultry is generally considered to be the most important single reservoir for campylobacters, mainly *Campylobacter jejuni*. However, there is some evidence based on the temporal occurrence of serotypes and genotypes shared by humans and poultry and on weekly data for poultry and human isolates that suggests that there is a common source of campylobacters instead of direct poultry-human transmission (28, 32). In addition, genotyping data on campylobacters of human and animal origin have raised the question of whether the role of poultry as a source of campylobacter infections has been overestimated (21, 40, 48).

Cattle are also common carriers of campylobacters (23, 25, 49). However, beef is not considered to be an important vehicle of transmission in human infections, because campylobacters are not commonly detected on carcasses or in beef. In surveys of retail beef only 0 to 5% of the samples have tested positive for campylobacters (42, 50, 55). Instead, the importance of raw milk as a risk factor for human campylobacteriosis has been recognized in epidemiological studies (33, 51), and consumption of unpasteurized milk has been associated with campylobacter infections in several outbreaks (12, 30, 47, 51). The environmental load of campylobacters in cattle manure may be a more significant factor in the transmission of infections than contaminated milk or beef (36, 39).

Antimicrobial treatment is not usually required for human campylobacter infections. In cases with severe or prolonged symptoms macrolides or fluoroquinolones have been recommended as treatment. Since the 1990s the increasing resistance of campylobacters to antibiotics, especially to fluoroquinolones, has been reported both among animal isolates and among isolates from human infections (10, 18). Because person-to-person transmission of campylobacters is uncommon and infections are frequently acquired from foods of animal origin, the use of antimicrobials in production animals has been suggested as the cause of the increase in resistance (3, 41). In Finland, products containing macrolides and fluoroquinolones are authorized for bovine use, but their use is limited.

The objective of the present study was to elucidate the role of Finnish cattle as a potential reservoir for thermophilic campylobacters and as a source of antibiotic-resistant *C. jejuni*.

MATERIALS AND METHODS

Sampling. Rectal fecal samples (n = 952) and carcass surface samples (n = 948) from clinically healthy cattle were collected in 12 slaughterhouses in Finland during the period January to December 2003. Sampling was carried out weekly, every second week, or every fourth week. The number of samples and the sampling frequency were calculated from the proportion of the slaughter volumes at each slaughterhouse in 2002. The samples were randomly chosen and taken by meat inspection veterinarians. The plastic sampling jars were filled with 200 to 300 g of fecal material and closed tightly, leaving the air space as small as possible. The carcass surface samples from the same animals were taken before chilling. The brisket, the inner and outer thigh, and the pelvic cavity were

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swabbed with two gauze pads (10 cm by 10 cm) wetted with sterile 0.1% peptone water. Both gauze pads were placed in a sterile plastic bag, the air was squeezed out, and the bag was closed tightly. All samples were sent chilled to the National Veterinary and Food Research Institute (currently the Finnish Food Safety Authority), Helsinki, Finland. The examination started in 1 to 2 days after sampling.

Isolation and identification of campylobacter strains. The fecal samples were examined by enrichment. Ten grams of fecal material was weighed and put into 90 ml of Bolton broth (Campylobacter Enrichment Broth, Lab 135 plus selective supplement X131 [LAB M, Bury, England] plus lysed horse blood) and incubated at 41.5°C for 24 h in a microaerobic incubator (ThermoForma [Thermo Electron Corporation, Marietta, OH]) (O_2, 5%; CO_2, 10%; N_2, 85%). One loopful (10 μl) of enrichment culture was spread onto modified *Campylobacter* charcoal differential agar (mCCDA) plates (Campylobacter Blood Free Selective Medium Lab 112 plus selective supplement X112 [LAB M, Bury, England]), which were incubated in the same conditions. In addition, one loopful (10 μl) of 730 fecal samples was directly cultured on mCCDA. The surface gauze samples were similarly enriched in 225 ml of Bolton broth and spread onto mCCDA.

Two colonies resembling campylobacters from mCCDA plates originating from direct culture and enrichment procedures were subcultured onto brucella agar (BBL, Becton Dickinson, MD) with 5% bovine whole blood treated with sodium citrate (Finnish Food Safety Authority, Helsinki, Finland). At least two isolates from each positive sample were identified to the species level using microscopical examination of motility and cell morphology, catalase and oxidase reactions, hippurate hydrolysis, and susceptibility to nalidixic acid (26). Nalidixic acid-resistant isolates were further examined for indoxyl acetate hydrolysis and susceptibility to cephalotin (26). Hippurate-negative, indoxyl acetate-hydrolyzing isolates were examined for H_2S production in triple sugar iron agar (LAB M, Bury, England) (pH 8) and for urease production to identify *Campylobacter hyointestinalis* strains. The isolates were stored in brucella broth supplemented with 15% glycerol at −70°C.

Serotyping. One to four *C. jejuni* isolates (287 in total) from 176 fecal samples and 21 isolates from carcass samples were serotyped using a set of 25 commercial antisera for the serotyping of heat-stable antigens (Penner) of *C. jejuni* by the passive hemagglutination method (Denka Seiken Co., Ltd., Tokyo, Japan). Tests were performed, and the results were interpreted according to the manufacturer's instructions.

Genotyping by PFGE. A total of 330 and 70 *C. jejuni* isolates from 183 fecal and 33 carcass samples, respectively, were analyzed using pulsed-field gel electrophoresis (PFGE). The agarose plugs were prepared according to the PulseNet protocol (www.cdc.gov/pulsenet/protocols) and stored in Tris-EDTA buffer at 4°C. DNA was digested overnight at 25°C with 20 U of SmaI restriction endonuclease (New England Biolabs Inc., Ipswich, MA) in a final volume of 200 μl containing 2 μl bovine serum albumin (New England Biolabs Inc., Ipswich, MA). PFGE was performed using the CHEF-DRIII pulsed-field electrophoresis system (Bio-Rad, CA). An agarose gel (1%) was prepared in 0.5× Tris-buffered EDTA (Sigma-Aldrich Co, Baltimore, MD). Fragments were separated by electrophoresis for 18 h at 6 V and 14°C with ramped pulse times from 6.8 to 35.4 s. *Salmonella* serotype Braenderup strain H9812 (ATCC BAA-664) was used as the fragment size marker. The gels were stained for 45 min with ethidium bromide (0.5 μg/ml) and photographed under UV illumination. Patterns that differed by at least a single band were considered to be different subtypes. Each subtype was named S1, S2, etc. The criteria presented by Tenover et al. (52) were used to assess how the subtypes were related.

Determination of antimicrobial susceptibility. The MICs of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline for 187 *C. jejuni* isolates from 183 rectal fecal samples were determined using a commercial broth microdilution method, VetMIC Camp (National Veterinary Institute, Uppsala, Sweden). Epidemiological cutoff values for resistance, based on MIC distributions, were used in the interpretation of results. A *C. jejuni* isolate was considered to be resistant to a specific antimicrobial when its MIC was distinctly higher than those of inherently susceptible *C. jejuni* isolates.

Statistical analysis. The χ^2 test (Excel; Microsoft Corp., Redmond, WA) was performed to investigate the association between month and prevalences of all campylobacters, *C. jejuni*, and *Campylobacter hyointestinalis* subsp. *hyointestinalis*.

RESULTS

Prevalence. Campylobacters were detected in a total of 296 out of 952 (31.1%) rectal fecal samples and in 33 out of 948 (3.5%) carcass surface samples. Campylobacters were detected in 4.4% and 37.4% of the fecal samples examined both by direct culture and by enrichment ($n = 730$), respectively.

TABLE 1. Distribution of campylobacter-positive animals between beef and dairy cattle farms

Herd type	No. of farms	No. of positive farms	% Positive farms	No. of positive animals
Beef cattle	284	122	42.7	154
Dairy cattle	463	133	28.7	142
Total	747	255	34.0	296

C. jejuni was detected in 186 (19.5%) and *Campylobacter coli* in 21 (2.2%) fecal samples. Presumptive *C. hyointestinalis* was isolated from 103 (10.8%) fecal samples, but the isolates from only 93 samples survived after storage at −70°C and all of these could be confirmed as *C. hyointestinalis* subsp. *hyointestinalis*. Two *Campylobacter* species were isolated from 14 samples: *C. jejuni* and *C. hyointestinalis* subsp. *hyointestinalis* in seven and *C. jejuni* and *C. coli* in six samples. The *C. coli* and *C. hyointestinalis* subsp. *hyointestinalis* isolates were detected only after enrichment. *C. jejuni* was detected in 29 (3.1%), *C. coli* in two (0.2%), and presumptive *C. hyointestinalis* in two (0.2%) carcass surface samples. In three cases the isolates from the fecal and carcass samples from the same animal represented different *Campylobacter* species.

Seventy percent of the animals belonged to the age group 1 to 3 years. In this age group the prevalences of *C. jejuni*, *C. hyointestinalis* subsp. *hyointestinalis*, and *C. coli* were 25.6%, 10.0%, and 1.9%, respectively. In the age group that included animals between 3 and 7 years, which represented 25% of the animals, the prevalences were 4.0%, 12.3%, and 3.1%, respectively.

The sampled animals were traced to 747 farms: 411 (43.2%) samples originated from 284 beef cattle farms and 541 (56.8%) samples from 463 dairy cattle farms (Table 1). The proportion of campylobacter-positive beef cattle farms was higher than that of dairy cattle farms. *Campylobacter* isolates originated from all of the 12 abattoirs and from 255 farms. More than one animal (two to five) per farm was sampled on 112 occasions. Animals from 19 farms were all campylobacter positive at the same sampling. In four cases, two *Campylobacter* species were detected in animals from the same farm. Positive and negative animals were detected from 36 farms on the same sampling occasion. Animals from 32 farms were sampled twice. Both samples from six farms were positive, and from 10 farms one of the samples was positive. Samples from 15 farms were campylobacter negative in both samplings. Two or more campylobacter-positive animals were detected from 33 farms either at the same sampling or on different occasions.

Monthly distribution. The monthly distribution of campylobacter-positive fecal samples is presented in Fig. 1. A slightly increasing trend can be seen in the overall prevalence of campylobacters towards the end of summer and late autumn. The prevalence of *C. jejuni* was highest in August and lowest in December. *C. hyointestinalis* subsp. *hyointestinalis* was most frequently isolated in November, and the lowest prevalence was detected in April. A statistical association was observed between month and the overall prevalence of campylobacters,

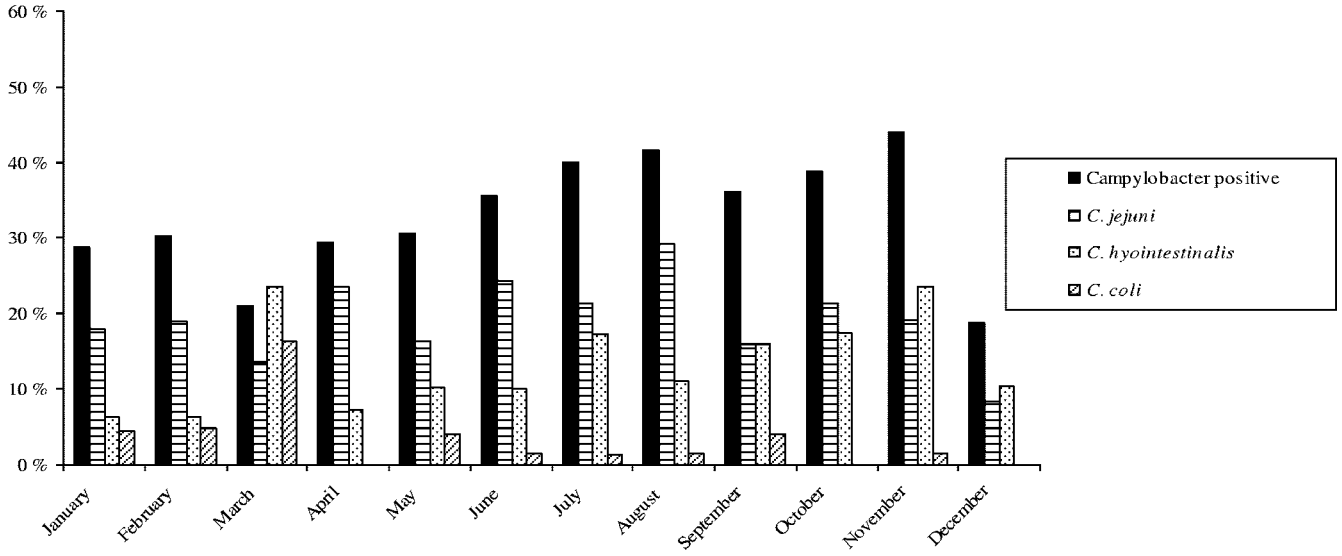

FIG. 1. Monthly distribution of campylobacters in the fecal samples.

but not *C. jejuni* ($P < 0.05$, df = 11). Also the prevalence of *C. hyointestinalis* subsp. *hyointestinalis* was statistically connected with month ($P < 0.01$, df = 11).

Serotyping. Seventeen different serotypes were detected among the fecal *C. jejuni* isolates using the commercial serotyping kit that was employed in this study. Untypeable isolates were obtained from 22 samples (12.5%). The predominant Penner serotypes of *C. jejuni* that were detected in the fecal samples were Pen2 and Pen4-complex, which were isolated from 52% of the fecal samples. In the 79 samples from which two or three isolates were serotyped, the same serotype was detected in 28 samples, two serotypes were detected in nine samples, and three were detected in one sample. Both typeable and untypeable isolates were obtained from the same sample on 17 occasions. Two untypeable isolates were obtained from four fecal samples. In carcass samples Pen2 was detected in 11 and Pen4-complex was detected in 5 out of 21 isolates.

PFGE. Fifty-six different *C. jejuni* subtypes were identified by PFGE among 330 isolates from the fecal samples. The 10 most prevalent subtypes isolated from 103 fecal samples covered 56.3% of *C. jejuni*-positive samples (Table 2). *C. jejuni* isolates from 164 animals (89.6%) were assigned to 21 SmaI subtypes. Unique subtypes were isolated from 30 animals (16.4%). DNA from five isolates was not digestible with SmaI. Multiple isolates were genotyped from 106 fecal samples. Two unrelated SmaI subtypes were observed in six of them. Two and three different but possibly related isolates were observed in two fecal samples.

When several animals from one farm were sampled at the same time, *C. jejuni* isolates from animals originating from the same farm represented indistinguishable SmaI profiles on eight occasions. Closely related subtypes were identified on one occasion and possibly related subtypes on two occasions. Unrelated genotypes were observed in five cases. On the six occasions when positive samples were obtained from the farms that were sampled twice, the *C. jejuni* isolates represented unrelated subtypes.

The *C. jejuni* isolates from carcass surface samples represented 20 different SmaI subtypes. The most frequently isolated subtypes were S1 and S20, which were each detected in four carcasses. Subtypes S9 and S26 were observed in three carcasses. Eleven subtypes were detected only once. Two different SmaI subtypes were isolated from two carcasses. In one case the isolates were possibly related, and in the other case they were unrelated.

On 12 occasions indistinguishable *C. jejuni* PFGE types were detected in the fecal and carcass samples from the same animal. Indistinguishable subtypes isolated from one animal's fecal sample were detected in another animal's carcass sample at six samplings. In eight cases different *C. jejuni* subtypes were obtained from fecal and carcass surface samples on the same sampling occasion.

Sero-/PFGE types. Twenty-three different PFGE subtypes were observed among *C. jejuni* isolates classified as Pen2. The largest group, Pen2/S1, comprised isolates from 19 animals. Isolates belonging to Pen4-complex were split up into 13 PFGE subtypes. The most common was Pen4-complex/S2, which was isolated from 10 animals. The predominant sero-

TABLE 2. Most prevalent PFGE types of *C. jejuni* in fecal and carcass samples

PFGE type	No. of fecal samples	% of positive fecal samples	No. of carcasses	% of positive carcass samples
S1	20	10.9	4	12.1
S2	14	7.7	2	6.1
S3	14	7.7	0	0.0
S5	10	5.5	1	3.0
S6	5	2.7	2	6.1
S7	10	5.5	1	3.0
S9	5	2.7	3	9.1
S10	6	3.3	1	3.0
S11	10	5.5	2	6.1
S13	7	3.8	0	0.0
S14	7	3.8	1	3.0
S20	0	0.0	4	12.1
S26	2	1.1	3	9.1

TABLE 3. Predominant combined sero-/PFGE types of *C. jejuni* isolates from fecal samples

Penner serotype	SmaI subtype	No. of positive samples	% of all positive samples
2	S1	19	10.8
2	S5	10	5.7
2	S11	7	4.0
2	S18	5	2.8
4-complex	S2	10	5.7
4-complex	S3	7	4.0
12	S7	8	4.5
1,44	S6	5	2.8
Total		71	40.3

types/PFGE types are represented in Table 3. Pen2/S1 was also the most common type among isolates from carcass samples comprising isolates from four carcasses. Pen2/S9, Pen2/S34, Pen4-complex/S2, and Pen1,44/S26 were all detected in two carcasses.

Antimicrobial susceptibility of *C. jejuni*. Of the 187 *C. jejuni* isolates that were examined for antimicrobial susceptibility, 16 (9%) were resistant to at least one of the antimicrobials tested (Table 4). Resistance to nalidixic acid was most commonly detected. Six of the 11 nalidixic acid-resistant isolates were also resistant to enrofloxacin. No multiresistance was observed among the isolates.

DISCUSSION

The prevalence of *Campylobacter* spp. in Finnish cattle at slaughter varied monthly between 18.8% and 44.1% during this 1-year study. In several studies performed in other countries prevalences of between 7% and 100% at slaughter have been reported (2, 5, 14, 37, 42, 49). Due to the different study designs regarding various sampling methods and materials, detection methods, etc., the results are not always comparable. The sampling for our survey was carried out in 12 out of 15 Finnish slaughterhouses, which covered 98% of the cattle slaughtered in Finland in 2003. The prevalence of campylobacters in cattle was 4.4% by direct culture and 37.3% by enrichment from the same 730 rectal fecal samples, which suggests that the overall level of campylobacters in the intestinal contents of cattle in Finland was low. This result is in accordance with the reported average most probable number values between 69/g and 6.1×10^2/g in the fecal samples of dairy cattle from other studies (36, 49). Higher numbers of cells have been obtained using real-time PCR for the quantification of campylobacters (25).

The predominance of *C. jejuni* over other *Campylobacter* species has been reported in cattle by Nielsen et al. (37) and by Açik and Çetinkaya (2) and many other studies, whereas *C. hyointestinalis* was the species that was most frequently isolated from cattle at slaughter in the surveys by Grau (17) and Pezzotti et al. (45). In the present study, *C. jejuni* was the most common species in young animals, while in the older age group *C. hyointestinalis* subsp. *hyointestinalis* was most frequently detected. A similar distribution of the *Campylobacter* species among young and adult cattle was reported by Giacoboni et al. (15). In addition to the age of the animals, the choice of method can also influence the diversity of the *Campylobacter* species detected from the samples. In our study, no *C. coli* or *C. hyointestinalis* subsp. *hyointestinalis* isolates were obtained by direct culture. The actual prevalence of *C. hyointestinalis* subsp. *hyointestinalis* in Finnish cattle is probably even higher than observed in this study, where the culture medium and growth conditions were optimized for the selection of the thermophilic *Campylobacter* species. These cultivation methods also exclude more fastidious species like *Campylobacter lanienae*, which proved to be the most prevalent *Campylobacter* species in beef cattle in the studies by Inglis et al. (24, 25), who employed PCR methods for detection.

Significant seasonal variation in the numbers of thermophilic campylobacters in dairy cattle herds but not in beef cattle has been reported by Stanley et al. (49). No evidence of the influence of climatic factors was observed, and the authors suggested that increased fecal excretion of campylobacters was due to hormonal factors or changes in the water supply and diet. In our study the overall patterns of monthly distribution of campylobacters in beef and dairy cattle were similar (data not shown). *C. jejuni* and *C. hyointestinalis* subsp. *hyointestinalis*, however, showed slightly different monthly patterns. The increasing prevalence of *C. jejuni* towards the end of the summer, although not significant, may reflect the continuous challenge during the grazing period (June to September) originating from environmental sources such as drinking water (20, 23) in contrast to the winter period, when the cattle are kept inside in Finland and given tap water to drink. No obvious reason could be found for *C. hyointestinalis* subsp. *hyointestinalis* reaching its highest level in November. In the Nordic countries, a seasonal peak in reported human campylobacter infections as

TABLE 4. Distribution of MICs among *C. jejuni* isolates

Antimicrobial	% Resistant isolates (95% CI[a])	Breakpoint for resistance (mg/liter)	Range of dilutions tested (mg/liter)	% of isolates with MIC[b] (mg/liter):													
				≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	1.6 (0.3–4.6)	>16	0.5–64					5.9	7.0	41.2	40.1	3.2	1.1	0.5	1.1		
Enrofloxacin	3.2 (1.2–6.9)	>0.5	0.03–4	1.1	8.0	49.2	33.7	4.8	1.1	0.0	1.6	0.5[c]					
Erythromycin	0 (0.0–2.0)	>8	0.12–16			1.1	1.6	22.5	51.9	20.9	2.1						
Gentamicin	0 (0.0–2.0)	>4	0.25–8				3.2	54.0	42.2	0.5							
Nalidixic acid	5.9 (3.0–10.3)	>16	1–128							1.6	15.5	61.0	16.0	3.7	0.0	0.5	1.6[c]
Tetracycline	1.1 (0.1–3.8)	>2	0.25–32				92.0	5.9	0.5	0.5	0.5	0.5					

[a] CI, confidence interval.
[b] MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.
[c] MIC greater than the highest concentration in the range of dilutions tested.

well as in the number of campylobacter-positive broiler flocks has consistently been observed in late summer (22, 35a, 40).

The proportion of campylobacter-positive cattle farms was low, on average 34%, in the present study. In a Danish study *C. jejuni* was present on 83% of dairy farms (36). The low number of samples per farm may explain the low percentage of positive farms in our study. Beef cattle were more frequently colonized by campylobacters than dairy cattle. A similar observation was reported by Beach et al. (5) and Grau (17), who suggested that the diet and high animal density of lot-fed cattle encouraged the intestinal colonization and spread of campylobacters. The variation in the colonization of beef and dairy cattle observed in our study may, however, reflect the age of the animals rather than the type of the herd, because most of the beef cattle were slaughtered at the age of 1 to 2 years, whereas most dairy cattle were slaughtered between the ages of 3 and 7 years. A higher prevalence of campylobacters in young animals has been observed in other studies (17, 36).

The predominant Penner serotypes of *C. jejuni* observed in this study (Pen2, Pen4-complex, Pen1,44, and Pen12) were also common in the human infections originating in Finland during the period July to September 1999 (53), but the most prevalent serotype in the human cases originating in Finland, Pen6,7, was rarely observed in cattle. The Pen4-complex and Pen12 serotypes have also been reported in Finnish poultry, although Pen6,7 was predominant (44). The percentage of fecal samples that yielded untypeable isolates was 12.5%, which is in accordance with the results from other studies, where commercial antisera from the same manufacturer were used for serotyping (44, 46). In our study, Pen2 was most frequently isolated from cattle in June (data not shown), whereas Vierikko et al. (53) reported a peak in the occurrence of the same serotype in humans in Finland in August. It would be interesting to find out whether these two peaks really do follow each other, suggesting that cattle may play a role in human infections. These data, however, originate from different years, and the annual variation cannot be excluded. The second most prevalent serotype from bovine samples, Pen4-complex, showed a different seasonal pattern with a peak in September regarding cattle, but it was at its highest in humans in August (53). These two serotypes were also the most commonly detected in dairy cattle in other studies (9, 27, 36, 37), which suggests that they may be particularly adapted to colonizing the bovine gut. Cocolonization by two serotypes in 8% of animals was reported by Nielsen (36). In the present study concurrent colonization by two *C. jejuni* serotypes was observed in 39% of animals from which two or more isolates were serotyped, assuming that the untypeable isolates represent different serotypes from the identified serotypes in the same sample.

Genotyping by PFGE revealed a high degree of diversity among the bovine *C. jejuni* isolates. This has been seen in other studies with other typing methods as well (2, 6, 48) and also in regard to *C. jejuni* isolates from chickens, sheep, turkeys, water, and human cases (9, 13, 38). A wide variation of SmaI subtypes could be observed among the isolates representing the most common serotype, Pen2. This has been found previously in the Danish study by Nielsen (36). Genomic instability, which enables the adaptation of the organism to variable environmental conditions (19, 54), has been given as the explanation for the diversity. However, significant genomic stability and clonal lineages of certain *C. jejuni* serotypes from a variety of hosts and geographic areas have been reported (29, 31). Despite the small number of isolates from each sample, more than one SmaI subtype was identified from 12% of the fecal samples from which multiple isolates were genotyped. The presence of unrelated subtypes in the samples suggests that there may have been several sources of campylobacters on the farm.

Although the sampling was planned only for investigating the situation at slaughter, the tracing of the animals to their farm also made some considerations possible at the farm level as well. When more than one animal was sampled at a time per farm, most commonly undistinguished or closely related subtypes were isolated from *C. jejuni*-positive samples. The coexistence of two or three unrelated *C. jejuni* subtypes or different *Campylobacter* species in the samples from a farm was observed in few cases. These observations might suggest animal-to-animal transmission or one or a small number of common sources of contamination (6, 36). Closely related isolates were rarely detected on a farm, which may reflect either the genetic instability of the strains or the temporary colonization of the animals. An indication of the latter may also be the detection of campylobacter-positive and -negative samples from the same farms at the same sampling. The observation that only a portion of the animals are simultaneously colonized is possibly due to the intermittent excretion of campylobacters or low numbers of campylobacters in the samples (36).

Campylobacters were not detected in almost half the cases when animals from the same farms were sampled twice. When this and the low prevalence of campylobacters in cattle at slaughter in this study are taken into consideration, it may be possible that cattle farms which are always campylobacter negative do exist. On the other hand, it may also reflect low numbers of campylobacters in the fecal samples.

Campylobacter contamination rates of 0 to 25% of carcasses before chilling and 3% after chilling have been reported in other studies (5, 17, 34). Due to the sensitivity of campylobacters to oxygen and drying, air chilling reduces the contamination of the carcasses (16, 17, 43). In the present survey the contamination level of carcasses was low (3.5%) before chilling, which may reflect the low number of campylobacters in cattle feces but probably indicates good slaughter hygiene as well and suggests that contamination of beef at the retail level is very low. Obviously, during the slaughter process cross-contamination can originate from the feces of the same animal or different animals through the slaughterhouse environment or equipment. The *C. jejuni* serotypes most frequently isolated from carcasses were the same as those isolated from the feces. Comparison of the PFGE subtypes from fecal and carcass samples revealed, however, that some subtypes commonly detected in fecal samples were not isolated from carcasses. This may indicate variation between subtypes regarding tolerance to oxygen and drying. One of the most common subtypes in carcass samples was not, however, isolated in feces. It may be possible that subtypes exist which are poor competitors in the intestines but can survive in the conditions on the surface of the carcass.

The overall prevalence of antimicrobial resistance among bovine fecal *C. jejuni* isolates was low. A small proportion of *C. jejuni* isolates were resistant to ampicillin, tetracycline, and

enrofloxacin. Aminopenicillins, fluoroquinolones, and tetracyclines are used in the treatment of bovine infectious diseases in Finland. No resistance to erythromycin was detected, although macrolides are used in the treatment of bovine infections. Resistance to nalidixic acid was almost twice as common as resistance to enrofloxacin. Similar findings on the resistance of bovine campylobacters to quinolones have been described by Aarestrup et al. (1) and Englen et al. (11). Comparison with resistance data from other countries is complicated by variations in the methodologies and breakpoints that are used to classify the isolates as resistant. Breakpoints recommended for *Enterobacteriaceae* by CLSI (formerly NCCLS) have usually been applied in previous studies, as no internationally agreed clinical or epidemiological breakpoints for antimicrobial resistance of campylobacters have been available. In a recent publication by CLSI (8) criteria are presented for erythromycin (≥32 μg/ml), ciprofloxacin (≥4 μg/ml), and tetracycline (≥16 μg/ml). Interpretation of MICs according to these criteria would have yielded less than 5% total resistance among the bovine *C. jejuni* isolates in the present study, which is substantially lower than that reported from other European countries and the United States (1, 4, 7, 11).

In conclusion, the prevalence of campylobacters in Finnish cattle at slaughter was low and carcass contamination was rare in this survey, indicating that Finnish beef can be considered as a minor source of campylobacters for consumers. The antimicrobial resistance level among bovine *C. jejuni* isolates was also low, and multiresistance was not detected, which may be explained by the prudent use of antimicrobial agents for animals. However, the common occurrence of serotypes Pen2 and Pen4-complex in cattle indicates that there may be an indirect association with human infections.

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