Chickens and Cattle as Sources of Sporadic Domestically Acquired Campylobacter jejuni Infections in Finland

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A substantial sampling among domestic human campylobacter cases, chicken process lots, and cattle at slaughter was performed during the seasonal peak of human infections. Campylobacter jejuni isolates (n = 419) were subtyped using pulsed-field gel electrophoresis with Smal, and isolates representing overlapping types (n = 212) were further subtyped using KpnI for restriction. The Smal/KpnI profiles of 55.4% (97/175) of the human isolates were indistinguishable from those of the chicken or cattle isolates. The overlapping Smal/KpnI subtypes accounted for 69.8% (30/43) and 15.9% (32/201) of the chicken and cattle isolates, respectively. The occurrence of identical Smal/KpnI subtypes with human C. jejuni isolates was significantly associated with animal host species (P < 0.001). A temporal association of isolates from chickens and patients was possible in 31.4% (55/175) of the human infections. Besides chickens as sources of C. jejuni in the sporadic infections, the role of cattle appears notable. New approaches to restrict the occurrence of campylobacters in other farm animals may be needed in addition to hygienic measures in chicken production. However, only about half of the human infections were attributable to these sources.

The incidence of human enteric infections caused by campylobacters is highest in the summer months, showing a consistent peak at the end of July in Finland (www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2008/2008b09.pdf), as well as in other Nordic countries (16, 33). Almost 70% of campylobacter infections detected in July and August in Finland are domestically acquired, whereas the annual average proportion of domestic cases is about 30%, and most of them are caused by Campylobacter jejuni (30). The prevalence of campylobacters in Finnish broiler flocks peaks simultaneously with the human cases (7), and similar sero- and genotypes have been reported among human and poultry strains isolated in Finland and in other countries (5, 8, 21–23). Several epidemiological studies have identified the handling and consumption of raw or undercooked poultry meat as a major risk factor for campylobacter enteritis (for example, see references 18, 20, and 41), whereas opposite conclusions about the significance of the survival of these fragile organisms in beef is poor (39, 42).

In a Finnish study combining data from the multilocus sequence typing of campylobacters isolated from production animals and from epidemiological studies of human cases, significant associations emerged between certain sequence-type complexes from human infections and contact with cattle, the consumption of unpasteurized milk, or the tasting or consumption of raw minced meat (23).

The aim of this study was to investigate the contributions of poultry and cattle as sources of human C. jejuni infections in Finland by comparing over a limited time frame the macrorestriction profiles obtained from pulsed-field gel electrophoresis (PFGE) analysis of a geographically representative collection of C. jejuni isolates from domestically acquired sporadic human infections, chicken process lots, and cattle.

MATERIALS AND METHODS

Isolates. We studied a total of 419 isolates. Human C. jejuni isolates (n = 175) were collected from June to August 2003, during the seasonal peak of human cases. The isolates represented all domestic C. jejuni strains isolated in 9 of 25 clinical microbiology laboratories located in nine hospital districts across the country. They were isolated from the fecal samples of patients using modified charcoal cefoperazone deoxycholate agar. One isolate per patient was submitted to the National Public Health Institute (KTL); currently, the National Institute for Health and Welfare (THL) for further investigation, and an isolate was defined as domestic if the patient had no history of traveling abroad within 10 days before the onset of symptoms or 17 days before the specimen was taken. Only isolates from sporadic infections were included.

Bovine fecal (n = 186) and carcass (n = 15) isolates were obtained from...
samples of 952 cattle in a survey carried out by the National Veterinary and Food Research Institute (currently, the Finnish Food Safety Authority Evira) at 12 of 15 Finnish slaughterhouses in 2003 (13). Altogether, 71 of the bovine fecal isolates originated from dairy cattle and 115 from beef cattle. Because most of the isolates originated from different farms and because long-term carriage of the same genotype of C. jejuni in a herd was considered likely, fecal isolates over the entire year were included in the study. Isolates from 262 carcass samples taken only between May and August 2003 were included, because those isolated during the rest of the year could not have been associated with human infections during the summer.

Isolates from chickens (n = 43) were obtained from cecal samples taken at slaughter. Two of three Finnish broiler slaughterhouses participated in this study. All 955 process lots slaughtered between May and August 2003 were sampled. One loopful (10 μl) of cecal contents of three to five chickens from each process lot was directly cultured on modified charcoal cefoperazone deoxycholate agar. One isolate from each campylobacter-positive process lot was submitted to Evira for further investigation.

**Identification and genotyping of isolates.** The identification of isolates was based on standard biochemical tests (19). The human isolates were genotyped at THL and the bovine and chicken isolates at Evira by PFGE using SmaI for restriction as described by Hakkinen et al. (13).

All isolates representing overlapping SmaI subtypes were additionally subtyped using KpnI for restriction. DNA was digested for a minimum of 4 h at 37°C with 20 U of KpnI restriction endonuclease (New England Biolabs, Inc., Ipswich, MA). PFGE data were analyzed with Bionumerics V5.10 (Applied Maths, Kortrijk, Belgium) at 0.5% optimization and 1.0% tolerance. Patterns differing by at least a single band were considered different subtypes. Subtypes obtained by SmaI and KpnI restriction were named S1, S2, S3, etc., and K1, K2, etc., respectively.

**Evaluation of the temporal association among isolates.** The temporal association of the SmaI/KpnI subtypes among isolates from chickens and patients was evaluated using the criteria presented by Ka¨renlampi et al. (21). The human isolates were genotyped at THL and the bovine and chicken isolates at Evira by PFGE using SmaI for restriction as described by Hakkinen et al. (13).

Isolates from humans, 43 (30.8%) of 201 isolates from cattle represented SmaI subtypes indistinguishable from those of chicken or bovine isolates (Table 1). The combined type S6/K12 predominated among isolates from human patients (12%) and occurred in both chickens and cattle (Table 1; Fig. 1).

**Overlapping combined SmaI/KpnI subtypes accounted for 69.8% (30/43) and 15.9% (32/201) of the chicken and cattle isolates, respectively.** The occurrence of identical SmaI/KpnI subtypes with human C. jejuni isolates was significantly associated with animal host species (P < 0.001).

A total of 17 of the 71 (23.9%) fecal isolates from dairy cattle and 15 (13.0%) of the 115 fecal isolates from beef cattle represented the overlapping SmaI/KpnI subtypes with human isolates. The occurrence of identical SmaI/KpnI subtypes with

### RESULTS

We identified 109 different SmaI subtypes among the 419 C. jejuni isolates investigated. Forty-three subtypes were distinguished among the 175 isolates from human infections, 15 subtypes among the 43 isolates from chickens, and 61 subtypes among the 201 isolates from cattle (data not shown). Of these, 26, 10, and 36 occurred only once in human, chicken, and bovine samples, respectively; 18 isolates from humans and 1 from chickens were untypeable by SmaI.

Fourteen SmaI subtypes of C. jejuni (32.6% of all 43 human subtypes) representing 114 (65.1%) of 175 human isolates were indistinguishable from those of chicken or bovine isolates (Table 1). In total, 36 (83.7%) of 43 chicken isolates and 62 (30.8%) of 201 isolates from cattle represented SmaI subtypes shared with humans.

Further subtyping of 212 C. jejuni isolates (114 human, 36 chicken, and 62 cattle isolates), representing the 14 overlapping SmaI subtypes, with KpnI as a restriction enzyme yielded 44 subtypes, 17 of which were shared between human and animal isolates (Table 1). The combined type S6/K12 predominated among isolates from human patients (12%) and occurred in both chickens and cattle (Table 1; Fig. 1).

Of the combined SmaI/KpnI subtypes, 12 were present only in humans, 4 only in chickens, and 12 only in cattle. In total, the SmaI/KpnI profiles of 97 (55.4%) human isolates were indistinguishable from those of chicken or cattle isolates. The overlapping combined SmaI/KpnI subtypes accounted for 69.8% (30/43) and 15.9% (32/201) of the chicken and cattle isolates, respectively. The occurrence of identical SmaI/KpnI subtypes with human C. jejuni isolates was significantly associated with animal host species (P < 0.001).

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human C. jejuni isolates in cattle was not significantly related to herd type ($P = 0.056$). All bovine subtypes overlapping those of humans occurred among isolates from dairy cattle, with the exception of S22/K16, isolated only from beef cattle (Fig. 2).

A temporal association of the SmaI/KpnI subtypes among isolates from chickens and patients was possible in 55 (31.4%) of 175 human infections (Table 2). Isolates from 12 (6.9%) human infections temporally associated with chicken isolates represented SmaI/KpnI subtypes that failed to occur in cattle.

**DISCUSSION**

In this study, we compared the DNA macrorestriction profiles of C. jejuni isolates from domestic human infections, chickens, and cattle covering the whole of Finland over a time frame of three summer months with the aim of estimating the attribution of these animal sources to human infections. A total of 419 C. jejuni isolates were genotyped with PFGE using SmaI and KpnI as restriction enzymes.

The C. jejuni isolates from food production animals were collected from 12 cattle slaughterhouses and 2 chicken slaughterhouses, representing 98% of the cattle and 85% of the chicken slaughter volume in Finland in 2003, respectively. The human clinical C. jejuni isolates of domestic origin represented 54% of all isolates collected by 9 of 25 Finnish clinical laboratories during a three-month period from June to August 2003. The total number of campylobacter infections reported during the same time period in Finland was 1,281, including infections contracted abroad (http://www3.ktl.fi/stat/).

The summer months were chosen as the time period to examine because of the pronounced seasonality of human campylobacteriosis and because the proportion of domestically acquired human cases in Finland is highest during the summer months (23; www.ktl.fi/attachments/suomi/julkaisukit /julkaisusarja_b/2005/2005b13.pdf). Furthermore, the occurrence of campylobacter in Finnish chicken process lots and, consequently, in retail poultry meat peaks in July and August (7, 15, 24). A comparison of C. jejuni isolates from retail chicken meat would have focused specifically on the genotypes to which consumers are exposed. On the other hand, by sampling at slaughter, we could obtain samples from more than human C. jejuni isolates from domestically acquired human infections, chickens, and cattle in Finland between June and August 2003.

![Figure 1](http://aem.asm.org/)

**FIG. 1.** Distribution of 17 *Campylobacter jejuni* SmaI/KpnI subtypes among isolates from domestically acquired human infections, chickens, and cattle in Finland between June and August 2003.

![Figure 2](http://aem.asm.org/)

**FIG. 2.** Distribution of the most prevalent SmaI/KpnI subtypes of human *Campylobacter jejuni* isolates among fecal isolates from Finnish dairy and beef cattle.

**TABLE 2.** Temporal association between human and broiler *Campylobacter jejuni* isolates during the seasonal peak in Finland from June to August 2003

<table>
<thead>
<tr>
<th>SmaI/KpnI subtype</th>
<th>No. of human isolates temporally associated with isolates from positive broiler flocks/total no. of human isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
</tr>
<tr>
<td>S4/K29</td>
<td>0/0</td>
</tr>
<tr>
<td>S6/K12</td>
<td>0/0</td>
</tr>
<tr>
<td>S7/K1</td>
<td>0/1</td>
</tr>
<tr>
<td>S7/K2</td>
<td>0/0</td>
</tr>
<tr>
<td>S7/K3</td>
<td>0/2</td>
</tr>
<tr>
<td>S54/K10</td>
<td>0/0</td>
</tr>
<tr>
<td>S54/K11</td>
<td>0/0</td>
</tr>
<tr>
<td>S64/K19</td>
<td>0/0</td>
</tr>
<tr>
<td>S74/K4</td>
<td>0/0</td>
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<tr>
<td>S74/K5</td>
<td>0/0</td>
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<tr>
<td>S74/K7</td>
<td>0/0</td>
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<tr>
<td>S76/K20</td>
<td>0/0</td>
</tr>
<tr>
<td>S77/K30</td>
<td>0/0</td>
</tr>
<tr>
<td>S78/K6</td>
<td>0/0</td>
</tr>
<tr>
<td>Total</td>
<td>0/3</td>
</tr>
<tr>
<td>Total no. of human isolates per month</td>
<td>11</td>
</tr>
</tbody>
</table>
80% of all process lots during the sampling period and, therefore, probably a better overall view of the situation. As Nielsen et al. (31) observed, the same C. jejuni subtypes that colonize the intestines of chickens can be detected in retail samples of chicken meat. Due to the small number of cecal samples per process lot, we may have excluded some positive lots if the contamination rate of chickens in the process lot was less than 50%. In recent years, after the implementation of the Finnish campylobacter monitoring program for poultry in 2004, slightly higher prevalences (5.9 to 7.4%) of campylobacters have been reported during the summer months, probably due to the higher number of cecal samples (10 ceca per process lot [7]), than the prevalence in this study, which is in accordance with that previously reported by Perko-Mäkelä et al. (35). In general, the prevalence of campylobacters in Finnish chicken process lots is lower than in most other countries, where prevalences from 15% to 87% have been reported (7, 29, 38). The proportion of slaughtering by each slaughterhouse in the preceding year was taken into account in the randomized sampling of cattle (13). The bovine fecal isolates collected throughout the entire year were included in our study, as evidence suggests that the long-term excretion of the same C. jejuni genotypes occurs both in dairy herds (14, 26) and in the farm environment (8).

As in several previous studies that have used different genotyping methods (8, 11, 26, 31), we obtained a wide variety of different C. jejuni subtypes with PFGE typing using SmaI and KpnI restriction enzymes. All different SmaI subtypes among multiple isolates from each bovine sample were included in our study. However, more than one SmaI subtype was present in less than 10% of the samples from cattle (13).

A few SmaI/KpnI subtypes predominated among the human isolates; the five most frequently detected comprised 37% of all the human isolates. Two subtypes predominated among the chicken strains, accounting for 27% of the chicken isolates. Isolates representing the most prevalent bovine SmaI subtypes (13), except S1, underwent no further analysis using KpnI restriction, because no identical SmaI types occurred among the human isolates. The predominant SmaI subtype in cattle, S1, was divided into seven KpnI subtypes, indicating that bovine isolates may be more evenly distributed among different subtypes than those from humans and chickens. This may reflect the diversity of sources of campylobacters in different geographical areas of Finland, where cattle farms are situated all over the country and chicken production is concentrated in the western part. Kwan et al. (26) and French et al. (9) have previously shown that the transmission of C. jejuni genotypes occurs over distances of only ca. 1 km at maximum in farmland area.

In a study by Kärenlampi et al. (22), the degree of overlap was 61% between human and chicken isolates and 5.7% between human and bovine isolates. Our observation of a higher overlap between isolates from humans and cattle (15.9%) may be due to the higher number of bovine isolates in our study but may also indicate differences in the sources of infection between rural and urban areas. Our isolates were collected from across the country, excluding the capital city of Helsinki, and thus covered rural areas more extensively than did the human isolates analyzed by Kärenlampi et al. (22) from the Helsinki district in the southern part of Finland. As Ethelberg et al. (6) and Garrett et al. (10) have suggested, the relative importance of poultry as a source of campylobacters may be lower in infections among the rural population. However, a higher percentage of chicken isolates (69.8%), compared with that of bovine strains (15.9%), represented SmaI/KpnI subtypes detected in human infections in our study.

SmaI/KpnI subtypes of C. jejuni isolated from chickens and cattle, including shared subtypes, were detected in 52% and 42% of human cases, respectively. Gilpin et al. (11) reported a similar overlap between bovine isolates and human infections. A similar percentage of overlap between campylobacters from chickens and humans, but much higher (83%) between those from cattle and humans, was observed in a study by Nielsen et al. (31). In our study, subtypes shared by chickens and cattle were isolated in 40% of the human cases and could have originated from either of the two animal reservoirs or from some source common to all three of the hosts. Half of the human infections in our study could not be explained by these animal reservoirs, which may indicate the existence of additional sources for campylobacteriosis besides chickens and cattle, as has been suggested previously (2, 23). On the contrary, based on English data, Wilson et al. (40) estimated that meat production animals and poultry are the sources of campylobacters in 97% of sporadic infections.

Hopkins et al. (17) concluded that genotypically similar C. jejuni strains are rather able to colonize a range of hosts instead of being host specific. Besides the SmaI/KpnI subtypes shared by all three of the hosts in our study, seven C. jejuni subtypes were shared between only humans and poultry and three between only humans and cattle. These subtypes could represent human pathogenic genotypes adapted to chickens and cattle. On the other hand, numerous subtypes were identified among strains isolated only from cattle and some only from chickens but not from human infections. This observation reinforces previous suggestions that probably not all C. jejuni types are pathogenic to humans, but nonpathogenic host-specific types may also exist in animal carriers (8, 9, 17, 23, 27, 34). In addition, the most prevalent of the shared C. jejuni subtypes in cattle, S5/K27, was detected in only one patient. This type could represent subtypes that are adapted to a specific animal host and that only occasionally cause disease in humans.

The temporal distribution of isolates from human infections and the appearance of indistinguishable SmaI/KpnI subtypes in chicken process lots indicate that up to 31% of the human cases of campylobacteriosis could have been mediated by chickens during the study period. Kärenlampi et al. (21) have presented a similar estimate. C. jejuni isolates from 27 (15.4%) human infections not temporally related to chickens were indistinguishable from bovine isolates. Taking into account the three subtypes shared only between humans and cattle (S5/K27, S22/K16, and S66/K18), which occurred in 3.4% of the human cases, an estimated 19% of the Finnish human infections could have been caused by C. jejuni strains originating from cattle in the summer of 2003. This estimate should be considered with caution, however, because indistinguishable genotypes may also exist in other animal or environmental sources not included in this study. In addition, some of the human infections temporally associated with chicken isolates could also have been caused by similar bovine campylobacters. However, this study confirms the conclusion of several authors
from other countries (9, 10, 25, 26, 31, 32) that cattle, in addition to chickens, can be an important source of C. jejuni for human sporadic infections.

The low-level occurrence of campylobacters in bovine carcasses and beef has been reported in several retail and slaughterhouse surveys (13, 31, 38). Therefore, beef is generally not considered significant in the transmission of campylobacteriosis. Our results support this conclusion, as none of the C. jejuni strains isolated from bovine carcasses represented similar Smal/KpnI subtypes to those of human isolates during the summer of 2003. Direct contact with cattle, feocally contaminated drinking and swimming waters, and raw milk have been suggested as routes of occupational and recreational exposure of rural populations to bovine C. jejuni (6, 10, 11). Drinking dug-well water and swimming in natural waters have been identified as risk factors for domestically acquired human campylobacteriosis in Finland (37), and significant associations have been shown between particular sequence-type complexes and the consumption of unpasteurized milk (23). Most milk is delivered to dairies (ca. 97% in 2003), and the consumption of unpasteurized milk is low in Finland (http://www.matilda.fi/servlet/page?_dad=portal30&_schema=906&784_MATILDA.portal30&_dad=porta130&_schema=906&784_MATILDA _JULKAISUT.4484043.docid=906&784_MATILDA _JULKAISUT.4484043.version=1170260951). However, occasional failures in milking hygiene can lead to the contamination of milk by campylobacters and cause family outbreaks on dairy cattle farms (36). In Sweden, ruminant density has proven to be more important than poultry-related factors for human campylobacter infections in rural areas (32). The situation may be similar in Finland, where the prevalence of campylobacters in chickens is low (7, 35) and cattle are common carriers of campylobacters (13).

Due to our substantial sampling over a limited time frame, we could estimate the relative contribution of two well-known reservoirs of campylobacters, chickens and cattle, to human campylobacter infections in Finland during the summer of 2003. Although chickens can be considered the most important single source of C. jejuni in sporadic, domestically acquired infections, the contribution of cattle appeared notable. Due to overlapping subtypes among chicken and bovine isolates, sources of specific animal species cannot be directly connected to sporadic infections and in chicken samples from the Helsinki area. The situation may be similar in Finland, where the prevalence of campylobacters in chickens is low (7, 35) and cattle are common carriers of campylobacters (13).

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