

Longitudinal Study of Finnish *Campylobacter jejuni* and *C. coli* Isolates from Humans, Using Multilocus Sequence Typing, Including Comparison with Epidemiological Data and Isolates from Poultry and Cattle[∇]

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Received 23 June 2006/Accepted 27 October 2006

We describe a study on the application of multilocus sequence typing for the analysis of *Campylobacter jejuni* and *C. coli* isolates from human domestically acquired infections in the Helsinki-Uusimaa area of Finland in 1996, 2002, and 2003. In addition, isolates from poultry meat and fecal samples of cattle from the seasonal peak (July to September) in 2003 were included in the study. In total, 361 Finnish *C. jejuni* and *C. coli* strains were typed. Sequence type 45 (ST-45) (45%), ST-21 (21%), and ST-677 (11%) clonal complexes were the most prevalent. The ST-45 and ST-677 complexes were overrepresented in comparison with previous studies. The longitudinal study revealed an association between *C. coli* (ST-828 complex) infection and elderly patients (≥ 60 years). Analysis of exposure factors, determined by a previous case-control study conducted during the seasonal peak in 2002, revealed that the ST-48 complex was significantly ($P < 0.05$) associated with the tasting or eating of raw minced meat. New and unassigned STs were associated with swimming in natural bodies of water, whereas the ST-677 complex was related to drinking nonchlorinated water from a small water plant or water from natural sources. The ST-45 complex was associated with contact with pet cats and dogs. In 2003, ST-45 occurrence was significantly associated with poultry whereas ST-50 was associated with isolates from humans. In contrast, ST-53, ST-58, ST-61, and ST-883 were significantly associated with isolates from cattle. Further studies are needed to reveal the significance of the observed associations.

Campylobacter jejuni and *C. coli* are the most frequent causes of bacterial gastroenteritis in Finland (www.ktl.fi/ttr). The number of laboratory-confirmed cases has nearly doubled over a 10-year period (2,197 cases in 1995 to 4,002 cases in 2005). *Campylobacter* species are frequently isolated from a wide variety of sources, including poultry, cattle, pigs, sheep, cats, dogs, wild birds, and water. Most *Campylobacter* infections are sporadic, and the relative contributions of different sources of infection remain unknown. Risk factors for *Campylobacter* infection, as identified by case-control studies, include eating or preparing raw or undercooked meat, especially chicken meat, drinking unpasteurized milk or untreated water, contact with domestic animals and pets, and foreign travel (14, 18, 26). A recent case-control study (19) revealed swimming in natural bodies of water to be a novel risk factor. In addition, eating undercooked meat and drinking from dug wells were independent risk factors for domestically acquired *Campylobacter* infections in Finland (19).

Discriminatory typing methods for use in the study of the molecular epidemiology and population genetics of *Campylobacter* isolates are crucial to better understand the epidemi-

ology and ecology of the organism. Once such information is obtained, it could be used to develop intervention strategies to limit the numbers of human infections. Dingle et al. (6) developed a multilocus sequence typing (MLST) scheme for *C. jejuni*, which has been shown to be a valuable tool for studying the diversity and population genetics of *Campylobacter* isolates. The advantages of MLST over other molecular methods, such as pulsed-field gel electrophoresis, include transferability, standardized nomenclature, free access to the database on the Internet, and direct comparability of results between different studies. Recently, an MLST scheme utilizing the same loci previously described for typing *C. jejuni* was also described for *C. coli* (4).

Studies using molecular methods suggest that some animal host-adapted genotypes or clonal lineages may never or only rarely cause disease in humans whereas others may be common human pathogens with one or several potential food-borne sources (3, 16, 22). Host associations among some *C. coli* genotypes have also been suggested (17). The impact of the host and that of the bacterial strain on the disease outcome are still poorly understood.

The aims of this study were (i) the longitudinal investigation of the genetic diversity, as measured by MLST, among Finnish *C. jejuni* and *C. coli* strains collected from domestically acquired infections in 1996, 2002, and 2003; (ii) the study of the relationships of sequence types (STs) and ST complexes of the infecting strain with demographic characteristics of the pa-

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[∇] Published ahead of print on 3 November 2006.

tients and serotype and detailed epidemiological data obtained in a case-control study conducted in Helsinki, Finland, in 2002 (19); and (iii) the evaluation of the overlap of MLST profiles between isolates from humans and those from bovine feces and retail poultry meat during the seasonal peak in 2003.

MATERIALS AND METHODS

Bacterial isolates. *C. jejuni* and *C. coli* isolates from domestically acquired human sporadic cases of gastroenteritis were collected at Helsinki University Central Hospital Laboratory from January to December inclusive in 1996 ($n = 94$), 2002 ($n = 112$), and 2003 ($n = 101$). Of these, 94 (*C. coli*, $n = 2$), 111 (*C. coli*, $n = 2$), and 100 (*C. coli*, $n = 3$) isolates, respectively, were successfully typed by MLST. The strains isolated during the seasonal peak from July to September in 2002 ($n = 46$) had been included in the epidemiological case-control study described in detail by Schönberg-Norio et al. (19, 20). The questionnaire filled in by the patients detailed questions about demographic and clinical data, travel history, recreational water activity, food (>30 exposures), and milk and drinking water consumption as well as contact with pet animals and other domestic animals during the 2-week period prior to falling ill. In addition to isolates from humans, *Campylobacter* isolates were collected from chicken and turkey retail meat samples from the Helsinki area as well as bovine fecal samples from slaughterhouses all over the country by random sampling during the seasonal peak of 2003. A total of 233 retail meat samples (169 chicken, 58 turkey, and 6 mixed meat) were analyzed, of which 41 chicken and 4 turkey samples were positive for *Campylobacter* spp. Of these, 36 (*C. coli*, $n = 3$) were successfully typed by MLST. In total, 20 bovine fecal isolates were typed by MLST. *C. jejuni* and *C. coli* cultures were identified by growth characteristics, microscopic analysis, catalase and oxidase production and hippurate hydrolysis.

DNA isolation. DNA isolation was carried out as described previously (13). Briefly, cells were harvested from blood agar plates, washed with SET buffer (150 mM NaCl, 15 mM EDTA, 10 mM Tris-HCl, pH 8.0), and lysed with sodium dodecyl sulfate (0.5%) and proteinase K (100 μ g/ml) at 50°C for 2 h. Protein removal was done by phenol:chloroform:isoamyl alcohol (25:24:1) extraction followed by extraction with chloroform. DNA was precipitated using 3 M sodium acetate (pH 5.3) and ethanol. The resulting precipitate was washed with 70% ethanol and finally suspended in Tris-EDTA buffer (pH 8), treated with RNase (10 μ g/ml), and diluted to approximately 10 ng/ μ l.

Multilocus sequence typing. Multilocus sequence typing was performed according to Dingle et al. (6), using additional primers for *C. coli* as described by Dingle et al. (4) and for *C. jejuni* as described by Schouls et al. (21), as well as gly-S5 and gly-S7 described at the *Campylobacter* MLST website (<http://pubmlst.org/campylobacter/>). PCR amplicons were purified using MultiScreen PCR plates (Millipore, MA) and suspended in 50 μ l 0.1 \times Tris-EDTA buffer. Sequencing reaction mixtures consisted of 0.5 to 2 μ l BigDye Terminator v3.1 ready reaction mix (Applied Biosystems, CA), 1.5 μ l template, and 1.3 pmol primer per 20- μ l reaction mixture. Sequencing reaction products were purified using MultiScreen SEQ plates (Millipore). Alternatively, PCR amplification products were purified by 20% polyethylene glycol-2.5 M NaCl precipitation (8) and sequencing products by sodium acetate-ethanol precipitation (<http://pubmlst.org/campylobacter/>). Electrophoresis was performed on a 96-capillary Abi 3700 sequencer with POP5 polymer or an ABI PRISM 310 genetic analyzer with a POP6 polymer (Applied Biosystems, CA). Allele sequences were assembled using Staden Package (version 1.6.0) (24).

Data analysis. Trace files of new allele sequences and new allelic profiles were sent to the PubMLST database for assignment of allele and ST profile numbers as well as clonal complex. Clustering of sequence data was done with BioNumerics v 4.01 software (Applied Maths, Kortrijk, Belgium).

Statistical tests. Cross-tabulations and statistical tests were performed using SPSS software (version 10.0). Analysis of association of the ST and ST complex with source of isolation and individual exposure factors was carried out using the Pearson chi-square or Fisher's exact two-tailed test, as appropriate. Results were considered statistically significant for P values of ≤ 0.05 .

RESULTS

A total of 305 human (*C. coli*, $n = 7$), 20 bovine, 32 chicken (*C. coli*, $n = 3$), and four turkey isolates were successfully typed by MLST (Table 1). These strains represented 19 established ST complexes and 81 STs. Of the 81 STs, 32 were described in

the present study for the first time. Isolates belonging to the new STs included 34 human (11%), two chicken (6%), and one bovine (5%) isolate. Eleven (34%) of the new STs were unassigned to a previously identified ST complex. In spite of the high numbers of different STs and ST complexes, three major types, ST-45, ST-50, and ST-677, covered 53% of all isolates, and the three major complexes ST-45, ST-21, and ST-677 covered 76% of all isolates. For the ST-21 complex, ST-50 was the type most prevalent among the Finnish population.

C. coli strains clearly diverged from those of *C. jejuni* in the neighbor-joining tree constructed from the combined MLST results (86 to 89% sequence similarity). Intraspecies sequence similarities varied from 99.8 to 100% for *C. coli* and from 96 to 100% for *C. jejuni*. In *C. jejuni*, the ST-61 *uncA* allele 17, which is typical for *C. coli* strains (<http://eburst.mlst.net/>), was present. In contrast, the ST-1367 *tkt* allele 194 had only 93.5 to 94.6 and 85.6 to 86.3% sequence similarities with other *C. jejuni* and *C. coli* strains, respectively.

Fourteen strains (one isolate from chicken and 13 [4%] from humans) were identified as possible mixed *C. jejuni/C. jejuni* or *C. jejuni/C. coli* cultures due to the observation of secondary peaks in the chromatograms of one or more of the seven loci. One allelic profile per pure culture was obtained, although several colonies were screened for alleles in which the secondary peaks had been observed. Finally, unambiguous profiles could not be obtained for one isolate from chicken and two from humans.

Diversity of isolates from domestically acquired infections in 1996, 2002, and 2003. The most common ST complexes among the isolates from humans were ST-45 (44.6%), ST-21 (19.7%), and ST-677 (12.1%) (Table 1 and Fig. 1). The ST-22 complex was more prevalent in 1996 than in other years ($P = 0.00001$, χ^2 test using Yates' correction). In comparison, the ST-45 complex was most prevalent in 2002 ($P = 0.042$) and the ST-21 complex in 2003 ($P < 0.00001$). Furthermore, ST-22 ($P < 0.00001$) and ST-1944 ($P = 0.048$, χ^2 test using Yates' correction) were found only in 1996 whereas ST-48 ($P = 0.012$) and ST-538 ($P = 0.032$) were detected only in 2002. ST-50 was detected more often than expected in 2003 ($P < 0.00001$) but less often than expected in 1996 ($P = 0.0016$). Similarly, ST-230 was detected more often than expected in 2002 ($P = 0.011$) but less often than expected in 2003 ($P = 0.031$). The highest number of isolates was obtained in July and August in each sample year (Fig. 2). However, no clear distinctions between the occurrence of different STs or ST complexes during the different seasons could be made.

The association of the age and gender of the patients with the STs and ST complexes was evaluated (data not shown). The ST-828 complex, including six out of seven *C. coli* isolates, was associated with advanced age (≥ 60 years; $P = 0.002$, χ^2 test using Yates' correction). The patient age range for all of the clinical *C. coli* isolates was 19 to 80 years (mean, 55 years). No significant associations between the gender of the patients and either the ST or the ST complex of the isolates were found.

Association of ST and ST complex with epidemiological data and serotype in 2002. Fisher's exact test was used to evaluate the associations between ST and ST complex and those risk factors given in the questionnaire of Schönberg-Norio et al. (19). The ST-48 complex was significantly associated with the eating or tasting of raw minced meat (Table 2).

TABLE 1. ST complex, ST, and allele distribution among *C. jejuni* and *C. coli* isolates^a

ST complex	ST	Allele(s) ^a							Species	No. of isolates				
		<i>aspA</i>	<i>glnA</i>	<i>gltA</i>	<i>glyA</i>	<i>pgm</i>	<i>tkt</i>	<i>uncA</i>		Human			Cattle yr 2003 ^c	Poultry yr 2003 ^c
										Yr 1996	Yr 2002 ^b	Yr 2003 ^c		
ST-21	21	2	1	1	3	2	1	5	<i>C. jejuni</i>			1		
	50	2	1	12	3	2	1	5	<i>C. jejuni</i>	6	11 (4)	33 (25)	1	5
	53	2	1	21	3	2	1	5	<i>C. jejuni</i>				4	
	451	2	1	2	3	2	3	5	<i>C. jejuni</i>		1		1	
	883	2	17	2	3	2	1	5	<i>C. jejuni</i>	1	1		3	
	1943	2	1	1	3	2	226	5	<i>C. jejuni</i>	1				
	1948	2	71	12	3	2	1	5	<i>C. jejuni</i>	1				
	1949	2	1	5	3	2	25	5	<i>C. jejuni</i>	1				
	1952	2	1	12	3	1	1	5	<i>C. jejuni</i>		1			
	1969	2	234	2	3	2	1	5	<i>C. jejuni</i>		1	1		
ST-22	22	1	3	6	4	3	3	3	<i>C. jejuni</i>	10				
	1947	1	94	6	4	3	3	3	<i>C. jejuni</i>	2				
	1966	1	3	6	4	3	276	3	<i>C. jejuni</i>			1 (1)		
ST-45	11	48	7	10	4	1	7	1	<i>C. jejuni</i>	3	2 (1)			
	45	4	7	10	4	1	7	1	<i>C. jejuni</i>	23	31 (8)	31 (27)	2	22
	137	4	7	10	4	42	7	1	<i>C. jejuni</i>	3	8 (3)	6 (2)		
	230	4	7	41	4	42	7	1	<i>C. jejuni</i>	3	9 (6)			
	334	4	7	40	4	42	7	1	<i>C. jejuni</i>		1 (1)			
	538	4	7	10	4	42	25	1	<i>C. jejuni</i>		4 (2)			
	583	4	7	10	4	42	51	1	<i>C. jejuni</i>	1				
	1003	8	7	4	4	125	7	1	<i>C. jejuni</i>		2			
	1326	104	7	10	4	1	7	1	<i>C. jejuni</i>	2				
	1944	4	7	10	4	320	7	1	<i>C. jejuni</i>	3				
	1950	4	7	197	4	1	7	1	<i>C. jejuni</i>	1				
	1958	4	7	41	4	42	7	177	<i>C. jejuni</i>		1 (1)			
	1964	4	7	143	4	1	7	1	<i>C. jejuni</i>			1 (1)		
	1971	4	233	10	4	1	7	1	<i>C. jejuni</i>					1
1973	4	7	10	266	1	7	1	<i>C. jejuni</i>			1			
ST-48	48	2	4	1	2	7	1	5	<i>C. jejuni</i>		5 (3)			
	475	2	4	1	4	19	62	5	<i>C. jejuni</i>	2	1 (1)		1	
	918	2	4	1	4	19	1	5	<i>C. jejuni</i>	1				
ST-52	52	9	25	2	10	22	3	6	<i>C. jejuni</i>	1	1 (1)	1		1
ST-61	61	1	4	2	2	6	3	17	<i>C. jejuni</i>				2	
ST-206	206	2	21	5	37	2	1	5	<i>C. jejuni</i>					
	46	2	21	5	3	2	1	5	<i>C. jejuni</i>			1		
	122	6	4	5	2	2	1	5	<i>C. jejuni</i>		1			
	227	2	4	5	2	2	1	5	<i>C. jejuni</i>	1				
	572	62	4	5	2	2	1	5	<i>C. jejuni</i>	1				
ST-257	824	9	2	2	2	11	5	6	<i>C. jejuni</i>			1		
ST-283	283	2	7	40	4	42	51	1	<i>C. jejuni</i>					
	267	4	7	40	4	42	51	1	<i>C. jejuni</i>		2			1
	383	4	7	12	4	42	51	1	<i>C. jejuni</i>	1				
ST-353	353	7	17	5	2	10	3	6	<i>C. jejuni</i>	1				
	1953	7	17	2	2	86	3	1	<i>C. jejuni</i>		1			
ST-446	446	47	55	5	10	11	68	8	<i>C. jejuni</i>					
	450	47	55	5	10	11	48	8	<i>C. jejuni</i>	1				
ST-574	574	7	53	2	10	11	3	3	<i>C. jejuni</i>					
	305	9	53	2	10	11	3	3	<i>C. jejuni</i>			1		
ST-607	607	8	2	5	53	11	3	1	<i>C. jejuni</i>					
	1716	8	2	5	62	11	5	1	<i>C. jejuni</i>		1			
	1915	8	2	5	53	11	3	6	<i>C. jejuni</i>	1				

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TABLE 1—Continued

ST complex	ST	Allele(s) ^a							Species	No. of isolates				
		<i>aspA</i>	<i>glnA</i>	<i>gltA</i>	<i>glyA</i>	<i>pgm</i>	<i>tkf</i>	<i>uncA</i>		Human			Cattle yr 2003 ^c	Poultry yr 2003 ^c
										Yr 1996	Yr 2002 ^b	Yr 2003 ^c		
ST-658	658	2	4	2	4	19	3	6	<i>C. jejuni</i>			1 (1)		
	1395	1	4	2	4	185	3	6	<i>C. jejuni</i>	1				
	1967	2	198	2	3	19	68	6	<i>C. jejuni</i>			2 (2)		
ST-677	677	10	81	50	99	120	76	52	<i>C. jejuni</i>	12	11 (8)	5 (5)		
	794	10	81	50	87	120	76	52	<i>C. jejuni</i>	4	3 (3)	2 (1)		1
ST-692	692	37	52	57	26	127	29	23	<i>C. jejuni</i>					
	1954	37	106	4	131	127	29	23	<i>C. jejuni</i>		1			
ST-828	828	33	39	30	82	104	43	17	<i>C. coli</i>					
	854	33	38	30	82	104	43	17	<i>C. coli</i>					2
	1156	33	39	30	79	104	64	17	<i>C. coli</i>	1				
	1413	33	38	30	82	104	117	17	<i>C. coli</i>					1
	1585	33	39	30	82	189	43	17	<i>C. coli</i>			1		
	1625	33	39	30	82	104	47	139	<i>C. coli</i>		1			
	1946	53	39	30	82	104	44	17	<i>C. coli</i>	1				
	1957	33	39	65	79	104	35	17	<i>C. coli</i>		1			
1965	159	236	30	78	104	43	17	<i>C. coli</i>			1 (1)			
ST-1034	1034	2	61	4	64	74	25	23	<i>C. jejuni</i>					
	1951	2	61	4	64	321	7	23	<i>C. jejuni</i>	1				
	1956	2	59	4	27	128	25	23	<i>C. jejuni</i>		1 (1)			
ST-1287	1287	84	106	29	28	74	136	90	<i>C. jejuni</i>					
	945	84	106	29	28	136	1	35	<i>C. jejuni</i>		1 (1)	2 (2)		
ST-1332	1332	2	1	29	28	58	25	58	<i>C. jejuni</i>	1				
Unassigned	58	19	24	23	20	26	16	15	<i>C. jejuni</i>				5	
	586	1	2	42	4	98	58	34	<i>C. jejuni</i>	1				
	1080	10	2	107	137	120	76	1	<i>C. jejuni</i>	1				1
	1367	10	119	50	126	171	194	112	<i>C. jejuni</i>		2 (1)			
	1525	2	59	4	27	128	7	23	<i>C. jejuni</i>		1 (1)			
	1759	7	112	5	10	11	67	6	<i>C. jejuni</i>		2			
	1945	7	78	42	264	106	12	8	<i>C. jejuni</i>	1				
	1955	18	235	72	100	116	103	6	<i>C. jejuni</i>		1			
	1959	27	2	22	104	319	86	16	<i>C. jejuni</i>			1		
	1960	161	33	22	104	134	109	16	<i>C. jejuni</i>			1		
	1961	76	22	165	98	146	269	16	<i>C. jejuni</i>			1		
	1962	55	172	21	49	125	83	51	<i>C. jejuni</i>			1		
	1963	7	2	5	10	22	37	1	<i>C. jejuni</i>			1 (1)		
	1968	33	39	30	79	113	47	178	<i>C. coli</i>			1 (1)		
	1970	37	29	75	48	126	5	23	<i>C. jejuni</i>					1
1972	7	71	5	62	11	67	1	<i>C. jejuni</i>				1		
1974	160	84	27	267	11	3	5	<i>C. jejuni</i>			1			

^a Alleles and STs first described in this study are shown in boldface type.

^b Numbers in parentheses are numbers of isolates included in the epidemiological case-control study conducted during the seasonal peak in 2002 by Schönberg-Norio et al. (19, 20).

^c All isolates from cattle and poultry were collected during the seasonal peak from July to September, as were numbers of isolates given in parentheses for isolates collected from humans in 2003.

Additionally, all patients with ST-21 and ST-48 complex isolates reported having eaten minced beef meat whereas only half of the patients with ST-45 (55%) or ST-677 (50%) complex isolates had done so (data not shown). ST-52 (ST-52 complex) was associated with the only reported contact with cattle. Patients who reported drinking unpasteurized milk were infected by ST-52 (ST-52 complex), ST-230 (ST-45 complex), and ST-1958 (ST-45 complex) isolates. Isolates from patients who reported eating or tasting raw or undercooked chicken

meat were not associated with any particular ST but belonged to clonal complexes ST-21, ST-45, ST-48, and ST-677.

Patients infected with new and unassigned ST isolates had been swimming in natural bodies of water more often than expected (Table 2). These patients were less frequently hospitalized. The ST-677 complex was associated with drinking non-chlorinated water from a small water plant and water from natural sources (i.e., lake, spring, stream, etc.). Nine patients (82%) infected with ST-677 complex isolates were hospitalized

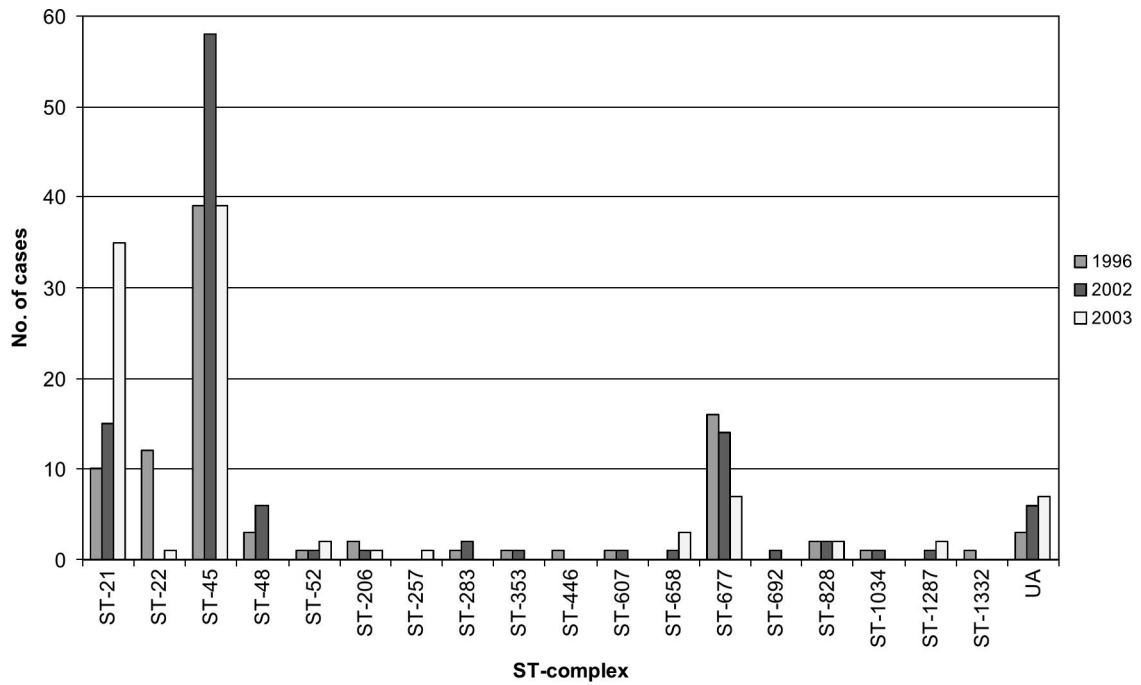


FIG. 1. Numbers of domestically acquired human campylobacteriosis cases per year among different ST complexes. UA, unassigned to a previously described clonal complex.

(data not shown). In comparison, 57% of all patients with *C. jejuni* and *C. coli* infections reported having been hospitalized. The difference in frequency of hospitalization among these patients was not statistically significant ($P = 0.082$). The patients with the ST-677 complex reported a mean length of stay in hospital of 3.9 days (average for all patients, 2.1 days). Among patients with ST-677 complex isolates, drinking from a dug well and contact with pet dogs were reported less frequently than among other patients (Table 2).

The ST-45 complex was significantly associated with contact

with cats and associated less frequently than expected with eating minced pork meat (Table 2). In addition, ST-45 was positively associated with contact with pet dogs and negatively associated with eating pork chops or boiled and otherwise cooked fish. On the other hand, ST-137 (ST-45 complex) was associated with eating soft cheese.

During the seasonal peak from July to September in 2002, Penner heat-stable (HS) serotypes of the isolates from humans were associated with the ST complexes as follows: HS1/44 with the ST-21 complex, HS4 complex with ST-48 and ST-677 com-

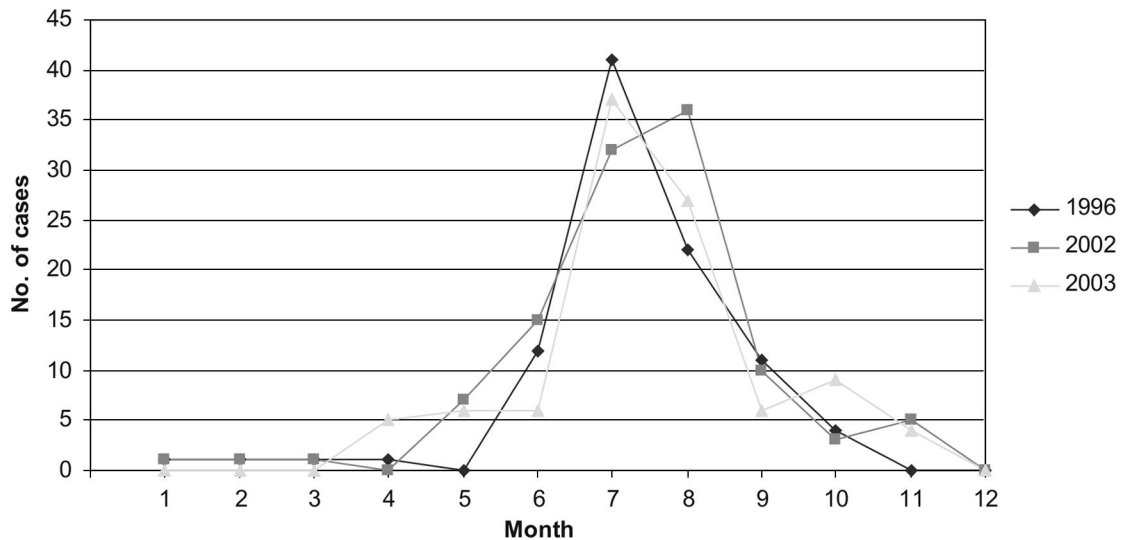


FIG. 2. Numbers of domestically acquired human campylobacteriosis cases per month during 1996, 2002, and 2003. Month 1, January; month 2, February; month 3, March; etc.

TABLE 2. Significant associations between ST or ST complex and epidemiological variables^a

Variable	No. of positive cases/ total no. of cases ^b	Association (no. of positive cases/ total no. of cases) ^b	P value(s)
Hospitalization	26/46	New and unassigned STs (0/4)	0.030
Swimming in natural bodies of water	18/46	New and unassigned STs (4/4)	0.019
Eating pork chops	15/36	ST-45 complex (0/6)	0.030
Eating minced pork meat	19/36	ST-45 complex (5/16)	0.042
Eating or tasting raw minced meat	2/38	ST-48 complex (2/3)	0.004
Eating boiled or otherwise cooked fish	21/42	ST-45 complex (1/8)	0.045
Eating soft cheese	15/40	ST-137 (3/3)	0.046
Drinking from a dug well	12/45	ST-677 complex (0/11)	0.023
Drinking nonchlorinated water from a small water plant	6/45	ST-677 complex (4/11)	0.025
Drinking water from natural sources	7/45	ST-677 complex (4/11)	0.050
Contact with a dog	23/44	ST-677 complex (2/10), ST-45 complex (7/8)	0.031, 0.048
Contact with a cat	14/38	ST-45 complex (10/18)	0.042

^a From the case-control study conducted by Schönberg-Norio et al. (19, 20).

^b Number of positive responses out of all responses to the variable in question.

plexes, HS5 with the ST-52 complex, and HS6/7 with the ST-45 complex. The ST-45 complex included many additional serotypes (HS12, HS21, HS27, HS55, and HS57) that were observed in one to three isolates (data not shown).

Diversity of isolates during summer peak in 2003. Figure 3 illustrates the percent distribution of isolates from humans, cattle, and poultry among the ST complexes during the seasonal peak from July to September in 2003. The distribution of STs of isolates from humans, cattle, and poultry is shown in Table 1. The levels of overlap of ST complexes between isolates from humans and cattle and between isolates from humans and poultry were 77% and 87%, respectively, whereas the respective levels of overlap of STs were 37 and 74%. ST-50 (ST-21 complex) was significantly associated with isolates from humans ($P = 0.00045$) and was less common among isolates from cattle than among other sources ($P = 0.0035$, Fisher's exact test). By comparison, ST-45 (ST-45 complex) was significantly associated with poultry ($P = 0.0028$, χ^2 test) and less common among isolates from cattle ($P = 0.002$). However, ST-53 (ST-21 complex) ($P = 0.00048$), ST-58 ($P = 0.00006$), ST-61 (ST-61 complex) ($P = 0.024$), and ST-883 (ST-21 com-

plex) ($P = 0.0035$) were significantly associated with isolates from cattle.

DISCUSSION

Our study is the first to describe, by use of MLST, Finnish *C. jejuni* and *C. coli* populations in humans and meat-producing animals. The strains used represented a spatially and temporally restricted collection, and 46 isolates from humans from the seasonal peak in 2002 had a known epidemiological background. The largest clonal complexes (ST-45, ST-21, and ST-677) were prevalent each year of the study and covered 76% of the isolates from humans. The clonal population structure may be due to niche adaptation, geographic isolation, host immune selection, or barriers to genetic exchange (23). Compared to previous studies using collections of strains obtained mainly from humans in the United Kingdom and The Netherlands (5) and also from diverse sources in the United Kingdom (16), we found ST-45 and ST-677 complexes to be more prevalent among the Finnish *Campylobacter* population. These results together with those of others (7, 10) suggest that different

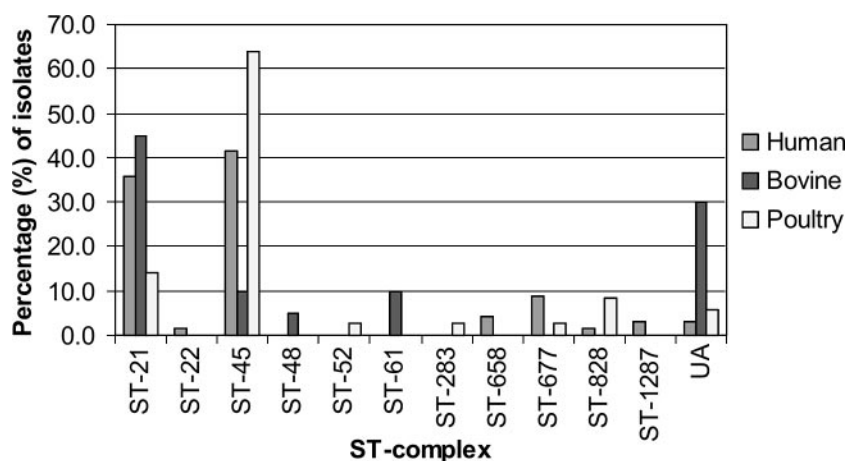


FIG. 3. Distribution of ST complexes among isolation sources during the seasonal peak from July to September in 2003. UA, unassigned to a previously described clonal complex.

genotypes may be more prevalent in different geographical areas.

The ST-828 complex (*C. coli*) was associated with patients of ≥ 60 years. This is in line with a previous report by Gillespie et al. (12), who showed that patients infected with *C. coli* tended to be older than those infected with *C. jejuni*. In our study, we found ST-854 (ST-828 complex) isolates in retail chicken meat; however, no overlap between chicken and human *C. coli* STs was seen. This result supports the view presented in a previous study (4) in which ST-854 was commonly found in chickens (29%) but not in pigs located on the same farm or in humans, suggesting that there might be a host preference for certain *C. coli* genotypes. The same study revealed a larger ST diversity among *C. coli* isolates from humans than among those from chickens and pigs. However, no overlap between isolates from these different sources was detected. In contrast, Miller et al. (17) identified most *C. coli* STs previously identified only in humans (4) also in samples from cattle, pigs, chickens, and/or turkeys, suggesting that many different transmission routes may play a role in the epidemiology of *C. coli*. Further studies should be conducted in order to understand the epidemiology of *C. coli* in more detail.

Drinking unpasteurized milk and contact with cattle have been implicated in *Campylobacter* infections in several studies (9, 18). In our study, ST-53, ST-58, ST-61, and ST-883 were significantly associated with isolates from cattle. In addition, ST-52 was associated with contact with cattle and drinking unpasteurized milk whereas ST-48 was associated with tasting or eating raw minced meat and ST-475 (ST-48 complex) was isolated from bovine feces. These results are in line with previous findings suggesting that ST-48 and ST-61 complexes are overrepresented in isolates from cattle (5, 11, 16). Furthermore, ST-58, which earlier was reported to occur only among cattle in Northern Ireland (<http://eburst.mlst.net/>), was identified only among isolates from cattle (25%) in our study. Further studies will confirm if ST-58 is associated with cattle and is uncommon in other sources as well as in human infections.

Eating or handling undercooked or raw chicken meat is a well-known risk factor for campylobacteriosis, as shown in several epidemiological studies (9, 14, 15, 18, 19, 26). In Finland, the production and consumption of chicken meat have almost doubled from 1995 to 2003 (<http://www.etl.fi/tilastot/pdf/myynti/Kotimaa2005.pdf>), and a similar increase has taken place in many other western European countries as well. A recent Danish epidemiological case-control study suggested that the increase in the consumption of fresh, unfrozen chicken meat since the middle of the 1990s has contributed to the increasing number of human *Campylobacter* infections (26). Our MLST results of isolates collected from humans in 1996, 2002, and 2003 support the expansion of certain clonal lineages, including a slight increase in the numbers of ST-45 and a more pronounced increase in ST-50, both types which often occur also among isolates from chicken. We found that ST-45 was the only type present in all sources studied in 2003. In our study, ST-45 was significantly associated with poultry and humans, in line with the findings of previous studies (2, 5, 16). Although we found that ST-45 was strongly associated with chicken, a variety of other STs were found in chicken meat as well. This result is in line with the finding that tasting or eating

raw and undercooked chicken meat was not associated with a particular ST or ST complex in our study.

Healthy cats and dogs are known to carry *Campylobacter* species, and contact with a pet dog and/or cat has been identified as a risk factor for human campylobacteriosis in previous studies (1, 15, 18, 27). In our study, the most common clonal complex, ST-45, was found to be significantly associated with contact with pet cats and dogs. It has previously been shown (25) that dogs that have regular contact with birds or poultry are more likely to carry *C. jejuni*. This is in line with our finding that the ST-45 complex was overrepresented among isolates from poultry. However, further studies on the importance of pet dogs and cats in human infections, especially in urban areas, are needed.

The ST-21 complex was the second largest clonal complex found in our study. It was predominated by ST-50 (74%), whereas only 2.7% of the strains represented the founder type, ST-21. Moreover, ST-50 was found to be overrepresented among isolates both from humans and from poultry. In other studies, the most abundant types that belonged to the ST-21 complex were ST-53 in The Netherlands (21), ST-21 in the United Kingdom and the United States (10, 16), and ST-262 and ST-53 in England (2). Although the ST-21 complex included isolates from all sources in our study, different STs were found to be predominant among isolates from cattle (ST-53 and ST-883) and isolates from humans and poultry (ST-50).

Another interesting finding was the association of ST-50 and ST-677 with isolates from humans. ST-677 was not detected among isolates from cattle or poultry in our study. Furthermore, ST-677 was more common among patients requiring hospitalization and a longer stay at the hospital, though further studies are needed to reveal if this particular clonal genotype could cause a more severe disease than others. Schönberg-Norio et al. (20) found that hospitalization due to *Campylobacter* infections was associated with advanced age. In our study, no statistically significant association was observed between the ST-677 complex and advanced age, although patients with the ST-677 complex had a mean age slightly higher than the overall average age of those with *Campylobacter* infection.

The occurrence of the ST-677 complex was associated with drinking from natural sources of water and nonchlorinated water from a small water plant. Previous findings of the ST-677 complex from wild birds and natural water sources support the possibility of enhanced survival and adaptation of this particular type for waterborne transmission. However, the negative association of the ST-677 complex with drinking water from a dug well and no association between it and swimming in natural bodies of water remain unexplained. However, the association of new and unassigned STs with swimming in natural bodies of water is in line with the finding of French et al. (11), who reported that the ST-45 complex and uncommon STs were overrepresented in the feces of wildlife and in natural bodies of water. In addition, many uncommon STs previously isolated only from wild birds in Sweden, including ST-1003, ST-1326, ST-1332, and ST-1367, were also isolated from patients with domestically acquired infections in Finland.

Several studies have addressed the questions of whether and to what extent *C. jejuni* populations in different food production animals and wildlife overlap with isolates from human

patients. Many STs have actually been shown to be shared by different sources. On the other hand, certain types that seem to be associated only with a particular source exist. Reasons for this might be due to differences in colonization potential and survival in the food processing chains or in the environment. Studies using MLST have revealed several globally common patterns but also some unique features for certain countries or geographical areas. Comparisons between different MLST types of *Campylobacter*, achieved utilizing microarrays, stress response analysis, survival, colonization potential, and infectivity, can be expected to reveal factors affecting the ecology and epidemiology of this important pathogen.

ACKNOWLEDGMENTS

This publication made use of the *Campylobacter jejuni* multilocus sequence typing website (<http://pubmlst.org/campylobacter/>) developed by Keith Jolley and Man-Suen Chan. The development of this site was funded by the Wellcome Trust.

This study was funded by the Academy of Finland, and R. Kärenlampi was supported by funds from the Finnish Graduate School on Applied Bioscience.

The City of Helsinki Environment Centre is acknowledged for providing the chicken and turkey isolates, and the National Veterinary and Food Research Institute of Finland is thanked for providing the bovine fecal samples.

REFERENCES

1. Carrique-Mas, J., Y. Andersson, M. Hjertqvist, Å. Svensson, A. Torner, and J. Giesecke. 2005. Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. *Scand. J. Infect. Dis.* **37**:101–110.
2. Colles, F. M., K. Jones, R. M. Harding, and M. C. J. Maiden. 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. *Appl. Environ. Microbiol.* **69**:7409–7413.
3. Devane, M. L., C. Nicol, A. Ball, J. D. Klena, P. Scholes, J. A. Hudson, M. G. Baker, B. J. Gilpin, N. Garrett, and M. G. Savill. 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *J. Appl. Microbiol.* **98**:980–990.
4. Dingle, K. E., F. M. Colles, D. Falush, and M. C. J. Maiden. 2005. Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. *J. Clin. Microbiol.* **43**:340–347.
5. Dingle, K. E., F. M. Colles, R. Ure, J. A. Wagenaar, B. Duim, F. J. Bolton, A. J. Fox, D. R. A. Wareing, and M. C. J. Maiden. 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. *Emerg. Infect. Dis.* **8**:949–955.
6. Dingle, K. E., F. M. Colles, D. R. A. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. L. Willems, R. Urwin, and M. C. J. Maiden. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. *J. Clin. Microbiol.* **39**:14–23.
7. Duim, B., P. C. R. Godschalk, N. van den Braak, K. E. Dingle, J. R. Dijkstra, E. Leyde, J. van der Plas, F. M. Colles, H. P. Endtz, J. A. Wagenaar, M. C. J. Maiden, and A. van Belkum. 2003. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curaçao, Netherlands Antilles. *J. Clin. Microbiol.* **41**:5593–5597.
8. Embley, T. M. 1991. The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. *Lett. Appl. Microbiol.* **13**:171–174.
9. Evans, M. R., C. D. Ribiero, and R. L. Salmon. 2003. Hazards of healthy living: bottled water and salad vegetables as risk factors for *Campylobacter* infection. *Emerg. Infect. Dis.* **9**:1219–1225.
10. Fitch, B. R., K. L. Sachen, S. R. Wilder, M. A. Burg, D. W. Lacher, W. T. Khalife, T. S. Whittam, and V. B. Young. 2005. Genetic diversity of *Campylobacter* sp. isolates from retail chicken products and humans with gastroenteritis in central Michigan. *J. Clin. Microbiol.* **43**:4221–4224.
11. French, N., M. Barrigas, P. Brown, P. Ribiero, N. Williams, H. Leatherbarrow, R. Birtles, E. Bolton, P. Fearnhead, and A. Fox. 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. *Environ. Microbiol.* **7**:1116–1126.
12. Gillespie, I. A., S. J. O'Brien, J. A. Frost, G. K. Adak, P. Horby, A. V. Swan, M. J. Painter, K. R. Neal, et al. 2002. A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerg. Infect. Dis.* **8**:937–942.
13. Harrington, C. S., F. M. Thomson-Carter, and P. E. Carter. 1997. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. *J. Clin. Microbiol.* **35**:2836–2892.
14. Kapperud, G., G. Espeland, E. Wahl, A. Walde, H. Herikstad, S. Gustavsen, I. Tveit, O. Natås, L. Bevanger, and A. Digranes. 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. *Am. J. Epidemiol.* **158**:234–242.
15. Kapperud, G., E. Skjerve, N. H. Bean, S. M. Ostroff, and J. Lassen. 1992. Risk factors for sporadic *Campylobacter* infections: results of a case-control study in southeastern Norway. *J. Clin. Microbiol.* **30**:3117–3121.
16. Manning, G., C. G. Dowson, M. C. Bagnall, I. H. Ahmed, M. West, and D. G. Newell. 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **69**:6370–6379.
17. Miller, W. G., M. D. Englen, S. Kathariou, I. V. Wesley, G. Wang, L. Pittenger-Alley, R. M. Siletz, W. Muraoka, P. J. Fedorka-Cray, and R. E. Mandrell. 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology* **152**:245–255.
18. Neimann, J., J. Engberg, K. Mølbak, and H. C. Wegener. 2003. A case-control study of risk factors for sporadic campylobacter infections in Denmark. *Epidemiol. Infect.* **130**:353–366.
19. Schönberg-Norio, D., J. Takkinen, M.-L. Hänninen, M.-L. Katila, S.-S. Kaukoranta, L. Mattila, and H. Rautelin. 2004. Swimming and *Campylobacter* infections. *Emerg. Infect. Dis.* **10**:1474–1477.
20. Schönberg-Norio, D., S. Sarna, M.-L. Hänninen, M.-L. Katila, S.-S. Kaukoranta, and H. Rautelin. 2006. Strain and host characteristics of *Campylobacter jejuni* infections in Finland. *Clin. Microbiol. Infect.* **12**:754–760.
21. Schouls, L. M., S. Reulen, B. Duim, J. A. Wagenaar, R. J. L. Willems, K. E. Dingle, F. M. Colles, and J. D. A. van Embden. 2003. Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. *J. Clin. Microbiol.* **41**:15–26.
22. Siemer, B. L., C. S. Harrington, E. M. Nielsen, B. Brock, N. L. Nielsen, J. Engberg, and S. L. W. On. 2004. Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. *J. Appl. Microbiol.* **96**:795–802.
23. Smith, J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt. 1993. How clonal are bacteria? *Proc. Natl. Acad. Sci. USA* **90**:4384–4388.
24. Staden, R., K. F. Beal, and J. K. Bonfield. 2000. The Staden package, 1998. *Methods Mol. Biol.* **132**:115–130.
25. Wieland, B., G. Regula, J. Danuser, M. Wittwer, A. P. Burnens, T. M. Wassenaar, and K. D. C. Stärk. 2005. *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. *J. Vet. Med. B* **52**:183–189.
26. Wingstrand, A., J. Neimann, J. Engberg, E. Møller Nielsen, P. Gerner-Smidt, H. C. Wegener, and K. Mølbak. 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. *Emerg. Infect. Dis.* **12**:280–284.
27. Workman, S. N., G. E. Mathison, and M. C. Lavoie. 2005. Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *J. Clin. Microbiol.* **43**:2642–2650.