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Decreasing Trend of Overlapping Multilocus Sequence Types between Human and Chicken *Campylobacter jejuni* Isolates over a Decade in Finland[▽]

C. P. A. de Haan,[1]* R. Kivistö,[1] M. Hakkinen,[2] H. Rautelin,[3,4] and M. L. Hänninen[1]

Department of Food Hygiene and Environmental Health, University of Helsinki, Helsinki, Finland[1]; Research Department, Finnish Food Safety Authority, Helsinki, Finland[2]; Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital Laboratory, Helsinki, Finland[3]; and Department of Medical Sciences, University of Uppsala, Uppsala, Sweden[4]

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We describe the long-term multilocus sequence typing (MLST) analysis of the population structure and dynamics of 454 Finnish human *Campylobacter jejuni* isolates, as well as 208 chicken isolates, collected during the mid-1990s to 2007. The sequence type clonal complexes (ST CC) ST-45 CC, ST-21 CC, and ST-677 CC were the most common ones found among all isolates, and they covered 73.9% of all isolates. The ST-283 CC also was found frequently among chicken isolates (8.2%). The predominant STs among all isolates were ST-45, ST-50, and ST-677. ST-137 and ST-230 were common among human isolates, and ST-267 was found more frequently among chicken isolates than human isolates. The ST-45 CC was significantly associated with chicken isolates ($P < 0.01$), whereas the ST-21 CC was associated with human isolates ($P < 0.001$). The ST-677 CC was not associated with any host ($P = 0.5$), and an opposite temporary trend of this complex was seen among chicken and human isolates, with an increase in the former and a decrease in the latter during the study period. Furthermore, the ST-22 and ST-48 CCs were significantly associated with human isolates ($P < 0.01$), but neither of the CCs was found in chicken isolates. The annual overlap between STs from human and chicken isolates decreased from 76% at the beginning of the study to 58% at the end. Our results suggest that the importance of chicken as a reservoir for strains associated with human infections has declined despite the consumption of domestic chicken meat increasing during the follow-up period by 83%.

Campylobacter jejuni is the most common bacterial cause of gastroenteritis worldwide. In Finland, registered human *Campylobacter* infections have increased from 2,629 cases in 1996 to 4,107 cases in 2007 (http://www3.ktl.fi/stat/). Of the 4,107 cases in 2007, 45% were registered in the Helsinki-Uusimaa region, where the annual incidence was highest (122/100,000; the national average is 78/100,000) as well. Most of the cases were associated with traveling to other countries. In 2007, approximately 57% of the patients had traveled outside of Finland prior to their illness, 19% had not traveled abroad, and for 24% information was unavailable (http://www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2008/2008b09.pdf).

In Finland, most of the *Campylobacter* infections are sporadic and appear during the summer months (http://www.efsa.europa.eu/en/scdocs/doc/130r.pdf). Between 1996 and 2007, approximately 45 to 55% of all registered infections were reported between June and September (http://www3.ktl.fi/stat/). In contrast to sporadic *Campylobacter* infections, outbreaks of campylobacteriosis are uncommon, usually occurring in spring or autumn, and are associated with drinking contaminated water (36).

Epidemiological studies performed in many countries indicate that handling or eating chicken meat is an important risk factor for the acquisition of campylobacteriosis (32, 46). A recent Finnish case-control study (39) identified swimming in lakes and rivers and drinking water from private wells as additional risk factors for acquiring the illness from domestic sources during the summer months. Contact with pets (19) and farm animals (21) and the consumption of raw milk (35) also have been identified as risk factors for *Campylobacter* infections.

The annual consumption of chicken meat in Finland increased by 83% (from 53 million to 95 million kg) from 1997 to 2007 but remained stable, at around 83.5 million kg, between 2002 and 2006. However, from 2006 to 2007, the consumption of chicken meat increased by a further 8% (from 88 million to 95 million kg). Most of the chicken meat consumed in Finland comes from domestic production (https://portal.mtt.fi/portal/page/portal/mtt/mtt/julkaisut/suomenmaatalousjamaaseutuelinkeinot/jul108_SM2008.pdf). Finnish chicken flocks have been monitored for *Campylobacter* spp. according to the regulations of the European Union since 2004 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF). Seasonality similar to that observed in human infections can be found in the prevalence of *Campylobacter*-positive chicken slaughter batches, i.e., 7.7% of all chicken slaughter batches were *Campylobacter* positive from June to October in 2007; however, during the rest of the year no positive chicken flocks were detected (http://www.efsa.europa.eu/en/scdocs/doc/130r.pdf).

To assess the relevance of a particular source and potential routes of transmission from animals to humans (6), the overlap

* Corresponding author. Mailing address: Department of Food Hygiene and Environmental Health, P.O. Box 66, FI-00014, University of Helsinki, Finland. Phone: 358919157456. Fax: 358919157101. E-mail: astrid.dehaan@helsinki.fi.

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between genotypes of *Campylobacter* isolates from patients and potential sources of infection has been studied using molecular typing techniques such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Of the two typing methods, PFGE is more discriminatory and therefore is considered more suitable for short-term epidemiological investigations and for the determination of the source of infection in outbreak situations (24, 26, 28).

In our previous studies, we have shown that during a seasonal peak, overlapping serotypes and PFGE genotypes exist between samples from patients suffering from campylobacteriosis and fecal samples from chicken (13, 17). Several other studies also have reported a 34 to 60% overlap between serotypes, genotypes, and/or sequence types (STs) of patient and poultry isolates using various typing techniques (20, 31, 37, 42).

Due to the high diversity of types and the lack of standardized nomenclature, PFGE is not a useful technique in long-term epidemiological studies. Unlike PFGE, MLST has been successfully used in long-term epidemiological studies (27, 45) and in deciphering the population structure (2, 20, 28, 45) of *Campylobacter* on a global scale. MLST has high discriminatory power (23) and standardized nomenclature for STs and clonal complexes (CCs) (3, 26).

The aim of our study was to analyze by MLST the overlap and dynamics of *C. jejuni* types among isolates collected from domestically acquired sporadic human infections from the Helsinki-Uusimaa region from 1996 to 2006 and isolates collected from domestic chicken production from 1999 to 2007.

MATERIALS AND METHODS

Bacterial isolates. The study included human clinical isolates only from domestically acquired sporadic *C. jejuni* infections in the Helsinki-Uusimaa region from January to December in 1996, 2002, and 2003 (18), from June to September in 1999 (17), and from July to December in 2006. All isolates were grown on modified charcoal cefoperazone deoxycholate agar (mCCDA) at 42°C for 48 h in a microaerobic environment created by a commercial gas-generating system (CampyGen; Oxoid, Basingstoke, Hampshire, United Kingdom). Chicken *C. jejuni* isolates were collected at three major Finnish slaughterhouses, which are responsible for all chicken meat production in Finland. In the scope of the Finnish campylobacter monitoring program, a pooled sample of 10 ceca was examined from all chicken slaughter batches from June to October in 2006 and 2007. In 1999, pooled contents of five ceca were examined from all chicken slaughter batches from May to September (34). A loopful (10 μl) of pooled cecal contents was cultured on mCCDA and grown in a microaerobic atmosphere created by a commercial gas-generating system (CampyGen; Oxoid, Basingstoke, Hampshire, United Kingdom) at 41.5°C for 48 h. From each positive sample one *C. jejuni* isolate was typed by MLST. In addition, the study included all 30 *C. jejuni* isolates from 233 fresh retail chicken meat samples of domestic origin from the Helsinki area collected between July and September in 2003 (18). These isolates were cultured on mCCDA (BBL, Becton Dickinson, MD) and incubated under microaerobic (5% O_2, 10% CO_2, 85% N_2) conditions at 41.5°C for 48 h in a microaerobic incubator. The amplification of the hippuricase gene as described by Shen et al. (40) was used for the confirmation of species of the isolates selected for the MLST study. All isolates were stored frozen at −70°C in skim milk with 20% glycerol after isolation.

DNA isolation. All isolates were grown on Brucella blood agar (5% bovine blood) prior to DNA isolation and incubated at 37°C for 24 h under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) in a microaerobic incubator. DNA was isolated using the Wizard genomic DNA purification kit (Promega, Germany) according to the manufacturer's instructions. DNA concentrations were measured by a Nanodrop ND-1000 (Thermo Scientific, Waltham, MA), diluted to 10 ng/μl, and stored at −20°C.

MLST. MLST typing was completed for a total of 454 *C. jejuni* human isolates and 208 *C. jejuni* chicken isolates (Table 1), including the human and chicken isolates from 1996, 2002, and 2003 typed in our previous study (18). Additional isolates from 1999, 2006, and 2007 were typed according to Miller et al. (30) or Korczak et al. (20). Briefly, 50 ng of DNA was mixed with 1× PCR buffer, 2.5 mM $MgCl_2$, 30 pmol of each primer, a 0.25 mM concentration of each deoxynucleoside phosphate, and 1 U of DyNAzyme polymerase. PCR conditions were 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C (35 cycles) or 30 s at 60°C and 2 min at 72°C (30 cycles) for primers described by Korczak et al. (20) or Miller et al. (30), respectively. When necessary, PCR conditions were adjusted for individual samples. For the purification of the PCR products, the MultiScreen PCR plates (Millipore, Billerica, MA) were used. Sequencing was carried out by the BigDye terminator v3.1 ready reaction cycle sequencing kit (Applied Biosystems, Foster City, CA). The annealing temperature was 51°C or 55°C for the Korczak et al. (20) and Miller et al. (30) primers, respectively. The Agencourt CleanSEQ kit was used for the purification of the reaction mixtures. The sequencing products were run on an ABI3130XL genetic analyzer or an ABI3730 DNA analyzer (Applied Biosystems).

Data analysis. Contig assembly and sequence editing were performed with Bionumerics version 5.1 software (Applied Maths, Belgium). For allele, sequence type (a combination of the seven different allele sequences), and clonal complex (a group of STs with four or more identical alleles) assignment, the PubMLST database was used (http://pubmlst.org/campylobacter/).

The Simpson's index of diversity (*D*) was calculated to determine and compare the ST diversity between human and chicken isolates (43). In the calculation, both the frequency and distribution of the genotypes are taken into account, resulting in a *D* value ranging from 0 (no diversity) to 1 (unlimited diversity). The more uniform the frequency and distribution, the greater the *D* value.

Population differentiation (Fst) between *C. jejuni* populations from humans and chickens was carried out with DnaSP (38). An Fst value of 0 indicates that the two populations are indistinguishable, whereas an Fst value of 1 indicates that the two populations are genetically distinct.

Linkage analysis (sI_A) and calculations on the genetic diversity of the populations were done with LIAN 3.5 (14). If there is a complete linkage equilibrium, which is indicative of a freely recombining population, then sI_A is equal to 0. If sI_A is significantly different from 0, then there is linkage disequilibrium, which is indicative of a clonal population structure.

The START2 package program was used to calculate the ratio of nonsynonymous to synonymous substitutions (*dN*/*dS*) in the seven different loci (15).

Fisher's exact two-tailed test or chi-square two-tailed test was used to assess associations between host and ST or CC. $P < 0.05$ was considered significant.

RESULTS

Diversity and overlap of STs of human and chicken isolates. Among the *C. jejuni* isolates studied ($n = 662$), 118 different STs emerged, 81 of which fell into 22 known CCs (Table 1). The most common CCs among the human isolates were the ST-45 (43.6%), ST-21 (19.4%), and ST-677 CCs (11.7%). Among the chicken isolates, the predominant CCs were the ST-45 (54.8%), ST-677 (9.6%), ST-21 (8.7%), and ST-283 CCs (8.2%). The most common STs among human isolates were ST-45 (28.0%), ST-50 (13.9%), ST-677 (9.3%), ST-137 (4.6%), and ST-230 (4.2%). Among chicken isolates, the prevailing STs were ST-45 (39.9%), ST-267 (8.2%), ST-677 (8.2%), and ST-50 (5.8%). The total overlap of STs in human and chicken isolates was 74.2% (Fig. 1). During the study period, a decrease in the overlap of STs from human and chicken isolates was observed. In 1999, the overlap between STs from human and chicken isolates was 76%; this declined to 68% in 2003 and further to 58% in 2006 (Fig. 2). The total decrease from 76% at the beginning of the study period to 58% at the end of the study period was statistically significant ($P < 0.01$). The overall diversity, calculated by the Simpson's index of diversity, of human isolates was greater ($D = 0.76$) than that of chicken isolates ($D = 0.68$). Fst values were calculated for the whole data set ($n = 662$) and for the years in which there were isolates from both hosts (1999, 2003, and 2006). For the whole data set the Fst value was 0.02583, and for 1999, 2003, and 2006 the values were 0.06025, 0.14441, and 0.04602, respectively.

TABLE 1. Major ST CCs and STs among *Campylobacter jejuni* isolates from human and chicken isolates between 1996 and 2007

Yr and isolate type (*n*)[a]	Total no. of CCs	Total no. of STs[b]	Human				Chicken			
			CC	%[c]	ST	%	CC	%	ST	%
1996										
Human (92)	13	31 (**28**)	ST-45 CC	42	ST-45	25				
					ST-11	3				
					ST-137	3				
					ST-230	3				
					ST-1944	3				
					Other	5				
			ST-677 CC	17	ST-677	13				
					ST-794	4				
			ST-22 CC	13	ST-22	11				
					ST-1947	2				
			ST-21 CC	11	ST-50	7				
					Other	4				
			ST-48 CC	3	ST-475	2				
					ST-918	1				
			Other	14						
1999										
Human (75)	8	24 (**19**)	ST-45 CC	45	ST-45	31	ST-45 CC	75	ST-45	64
					ST-230	8			ST-11	7
					ST-11	3			ST-230	4
					Other	3	ST-677 CC	7	ST-677	4
Chicken (28)	4	10 (**7**)	ST-677 CC	19	ST-677	16			ST-794	4
					ST-794	3	ST-21 CC	4	ST-883	4
			ST-21 CC	15	ST-883	9	ST-283 CC	4	ST-267	4
					ST-50	3	UA[d]	11	ST-693	4
					Other	3			ST-1080	4
			ST-283 CC	7	ST-267	5			ST-2186	4
					ST-383	1				
			ST-48 CC	4	ST-48	3				
					ST-4236	1				
			Other	10						
2002										
Human (109)	13	30 (**26**)	ST-45 CC	28	ST-45	28				
					ST-230	8				
					ST-137	7				
					ST-538	4				
					Other	6				
			ST-21 CC	14	ST-50	10				
					Other	4				
			ST-677 CC	13	ST-677	10				
					ST-794	3				
			ST-48 CC	6	ST-48	5				
					ST-475	1				
			ST-283 CC	2	ST-267	2				
			Other	12						
2003										
Human (97)	9	23 (**16**)	ST-45 CC	40	ST-45	32	ST-45 CC	73	ST-45	70
					ST-137	6			ST-1971	3
					Other	2	ST-21 CC	13	ST-50	13
			ST-21 CC	36	ST-50	34	ST-283 CC	3	ST-267	3
					Other	2	ST-677 CC	3	ST-794	3
Chicken (30)	4	7 (**5**)	ST-677 CC	7	ST-677	5	UA	6	ST-1080	3
					ST-794	2			ST-1970	3
			Other	17						
2006										
Human (81)	14	35 (**30**)	ST-45 CC	35	ST-45	23	ST-45 CC	48	ST-45	39
					ST-137	5			ST-583	5
					ST-583	2			Other	4
					Other	5	ST-283 CC	19	ST-267	19
			ST-21 CC	21	ST-50	14	ST-21 CC	11	ST-50	8
					Other	7			ST-451	3

Continued on following page

TABLE 1—*Continued*

Yr and isolate type (*n*)[a]	Total no. of CCs	Total no. of STs[b]	Human				Chicken			
			CC	%[c]	ST	%	CC	%	ST	%
Chicken (62)	5	16 (**10**)	ST-22 CC	11	ST-1947	9	ST-1287 CC	6	ST-945	6
					Other	2	ST-677 CC	5	ST-677	5
			ST-283 CC	6	ST-267	6	UA	10	ST-586	2
			ST-48 CC	4	ST-48	2			ST-1367	2
					ST-475	1			Other	6
			Other	23						
2007										
Chicken (88)	8	26 (**15**)					ST-45 CC	47	ST-45	23
									ST-3805	9
									ST-1326	6
									Other	9
							ST-677 CC	16	ST-677	15
									ST-794	1
							ST-21 CC	7	ST-50	3.5
									ST-53	3.5
							ST-283 CC	3	ST-267	3
							ST-383 CC	3	ST-356	3
							ST-1034 CC	3	ST-4001	3
							ST-1332 CC	3	ST-1332	3
							ST-692 CC	2	ST-1278	2
							UA	15	ST-3272	3
									ST-1367	2
									ST-4002	2
									Other	8

[a] Numbers in parentheses indicate the number of isolates typed by MLST per host per year.
[b] Numbers in boldface are the numbers of STs that belong to a known ST complex.
[c] Percentages shown after ST complexes and STs are the amounts of isolates of that particular year/host carrying that particular ST complex or ST.
[d] UA, unassigned (STs do not belong to any known CC).

Both sI_A values and genetic diversities (*H*) were calculated for the whole data set ($n = 662$), as well as for all human ($n = 454$) and all chicken ($n = 208$) isolates. The sI_A values were 0.7258 for the whole data set, 0.7111 for the human isolates, and 0.7528 for the chicken isolates. Values for the genetic diversity (*H*) for the whole data set, all human isolates, and all chicken isolates were 0.7196 ± 0.219, 0.7421 ± 0.216, and 0.6461 ± 0.0240, respectively.

Allelic diversity between human and chicken isolates. A total of 234 different alleles were found across the seven loci, of which 30 were identified for the first time either in the present study or by Kärenlampi et al. (18) and submitted to the

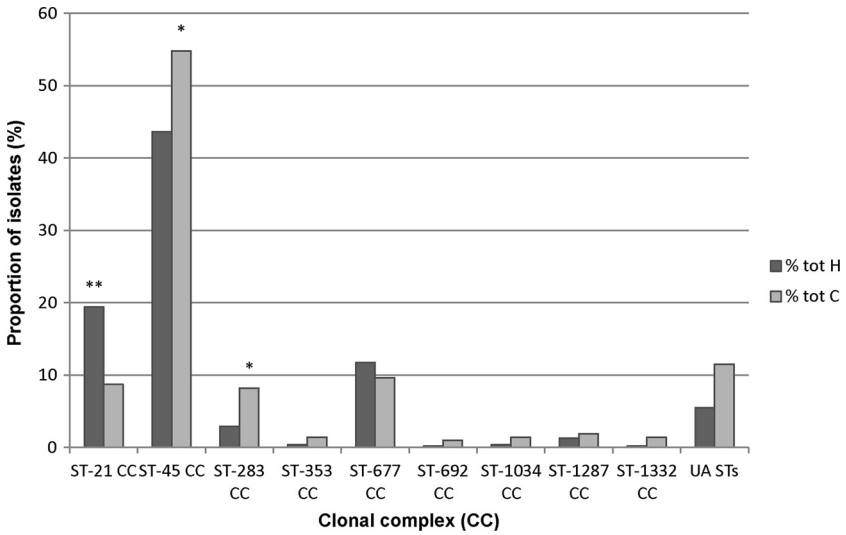

FIG. 1. Overview of the distribution of overlapping ST complexes between human (H) ($n = 454$) and chicken (C) ($n = 208$) isolates from 1996 to 2007 (shown as percentages). UA STs are STs that are not assigned to any known CC. Significant host associations are indicated with asterisks, where one asterisk indicates $P \leq 0.01$ and two asterisks indicates $P \leq 0.001$.

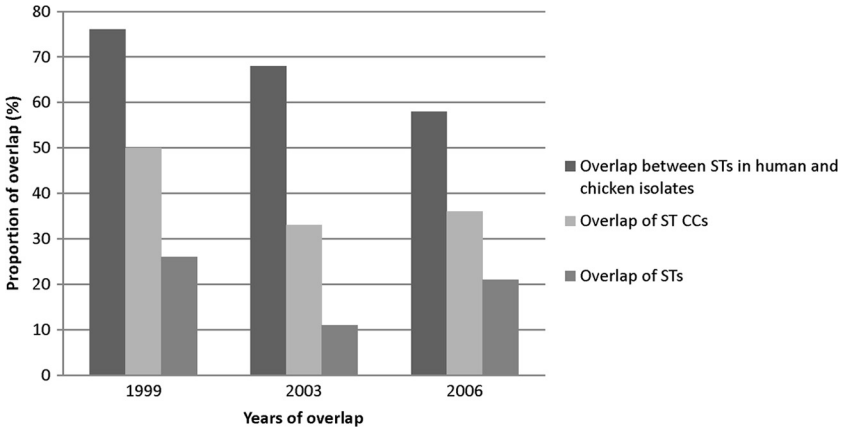

FIG. 2. Overview of the percentage of overlapping *C. jejuni* STs/CCs between human and chicken isolates in 1999, 2003, and 2006.

PubMLST database. The *uncA* locus was the least diverse, with 21 different alleles, and the *pgm* locus was the most diverse, with 41 different alleles. Generally, higher numbers of different alleles at each locus were found in the human isolates compared to those of the chicken isolates, but the *dN*/*dS* ratios were greater for the chicken isolates than for the human isolates (except for *aspA* and *uncA*) (Table 2).

Temporal and host associations with STs and CCs. Although ST-45 accounted for 20% to 70% of the MLST types of the chicken isolates during the study years, there was a sharp decrease in the prevalence of this ST among the chicken isolates between 2003 and 2006, as well as between 2006 and 2007 (Fig. 3a). Between 2003 and 2006, the ST-45 CC showed the same trend as ST-45 but was stable between 2006 and 2007 (Fig. 3b). Among the human isolates, the percentages of ST-45 (23% to 36%) and the ST-45 CC (35% to 53%) fluctuated as well; however, no trend similar to that in chicken isolates was detected. The ST-45 CC and ST-45 and ST-3805, which are found within this CC, were significantly associated ($P < 0.01$) with the chicken isolates. Similarly, ST-137 ($P < 0.01$) and ST-230 ($P = 0.03$) were significantly associated with the human host.

The ST-21 (ST-50), ST-22 (ST-22; $P = 0.02$), and ST-48 CCs were significantly associated ($P < 0.01$) with the human isolates. ST-50 was the most common ST in the ST-21 CC overall, and a peak occurrence of this ST was observed in humans in 2003 (Fig. 4a). Only in 1999 was ST-883 the most commonly found ST in the ST-21 CC in both hosts. The ST-22 CC was one of the most commonly detected CCs among the human isolates in 1996 (13.0%) and 2006 (11.1%) but not in other study years, except for one isolate from 2003 (Fig. 4b). Overall, the ST-48 CC was present in 3.3% of the human isolates, although not in 2003. The ST-22 and ST-48 CCs were not observed in the chicken isolates.

In human infections, a decreasing trend of the ST-677 CC isolates was found during the study period (Fig. 4c); however, in chickens, an increase was seen after 2003 (Fig. 3c). Overall, 10.7% of the human and chicken isolates belonged to the ST-677 CC. However, this complex was not associated with either host ($P = 0.5$), nor was ST-677 ($P = 0.7$) or ST-794 ($P = 0.6$), which are members of this CC.

The ST-283 CC was significantly associated with chicken isolates ($P < 0.01$), and a peak in the occurrence of the ST-283 CC was observed in the chicken isolates in 2006 (Fig. 3d). In human isolates, peak occurrences of the ST-283 CC were observed in 1999 and 2006 (Fig. 4d).

DISCUSSION

Our study is one of the first to describe MLST types and their distribution in human and chicken *C. jejuni* isolates over a decade, starting from the middle of the 1990s, when the numbers of registered *Campylobacter* infections began to increase in most European countries (http://www.efsa.europe.eu/en/scdocs/doc/130r.pdf). Simultaneously, chicken meat production and consumption have increased, which have been

TABLE 2. Allelic diversity among human ($n = 454$) and chicken ($n = 208$) isolates

Locus	No. of alleles (new alleles) in human isolates	No. of alleles (new alleles) in chicken isolates	*dN*/*dS*		
			Human isolates	Chicken isolates	Complete data set
aspA	23 (2)	15 (1)	0.0507	0.0288	0.0495
glnA	34 (3)	19 (3)	0.0526	0.1007	0.0607
gltA	27 (2)	22 (3)	0.0238	0.0291	0.0293
glyA	27 (3)	22 (2)	0.0519	0.0566	0.0529
pgm	38 (3)	17 (1)	0.0378	0.0407	0.0372
tkt	32 (3)	19 (3)	0.0357	0.0446	0.0393
uncA	19 (1)	13	0.0061	0.0000	0.0060 (0.000)[a]

[a] The *dN*/*dS* value in parentheses was calculated by the exclusion of the data for *uncA* allele 17, which comes from a different *Campylobacter* species.

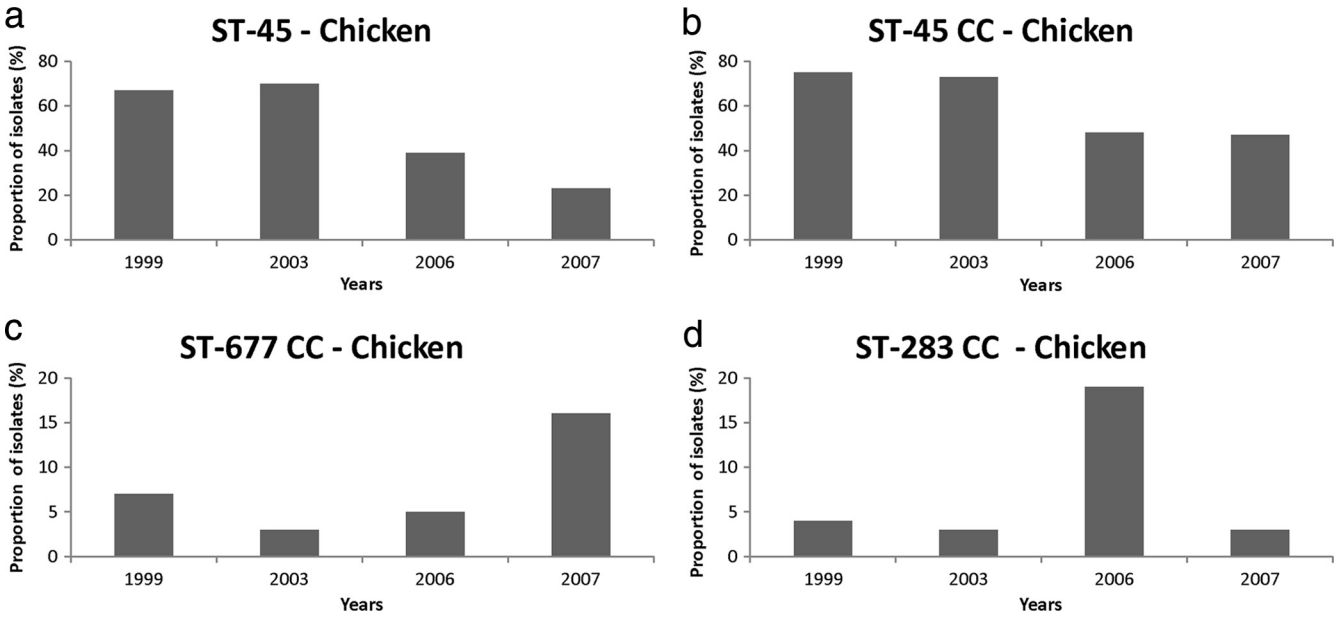

FIG. 3. Trends of MLST types in chicken isolates from 1999 to 2007. (a) Trend of ST-45 among chicken isolates from 1999, 2003, 2006, and 2007 shown as percentages of isolates with ST-45 in each year. (b) Trend of the ST-45 complex among chicken isolates from 1999, 2003, 2006, and 2007 shown as percentages of isolates with the ST-45 complex in each year. (c) Trend of the ST-677 complex among chicken isolates from 1999, 2003, 2006, and 2007 shown as percentages of isolates with the ST-677 complex in each year. (d) Trend of the ST-283 complex among chicken isolates from 1999, 2003, 2006, and 2007 shown as percentages of isolates with the ST-283 complex in each year.

linked to the rising numbers of human infections in other countries (44, 46). We investigated the sequence types of domestically acquired sporadic human *C. jejuni* infections in the Helsinki-Uusimaa hospital district from 1996 to 2006 and their potential association with *C. jejuni* isolates from chickens. This region is the most urbanized part of Finland and includes Helsinki, where the average campylobacteriosis rate is high compared to that of other parts of the country

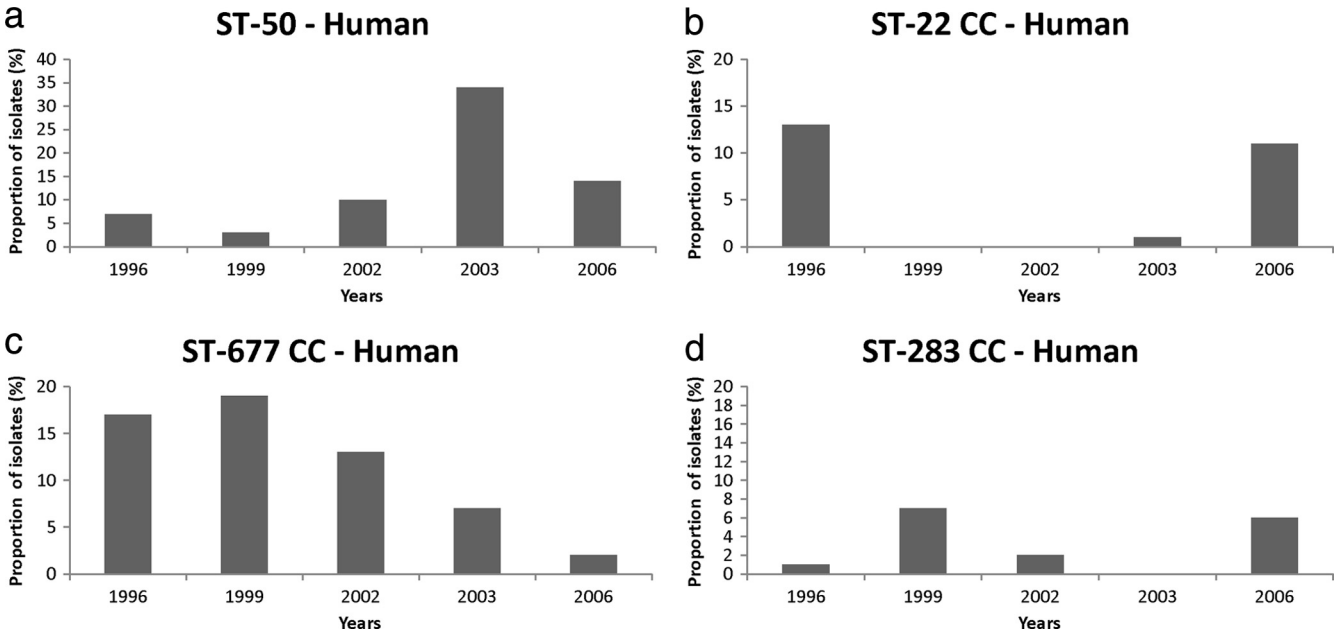

FIG. 4. Trends of MLST types in human isolates from 1996 to 2006. (a) Trend of ST-50 among human isolates from 1996, 1999, 2002, 2003, and 2006 shown as percentages of isolates with ST-50 in each year. (b) Trend of the ST-22 complex among human isolates from 1996, 1999, 2002, 2003, and 2006 shown as percentages of isolates with the ST-22 complex in each year. (c) Trend of the ST-677 complex among human isolates from 1996, 1999, 2002, 2003, and 2006 shown as percentages of isolates with the ST-677 complex in each year. (d) Trend of the ST-283 complex among human isolates from 1996, 1999, 2002, 2003, and 2006 shown as percentages of isolates with the ST-283 complex in each year.

(http://www3.ktl.fi/stat/). Although the total number of registered *Campylobacter* infections rose by 56% during the study period (http://www3.ktl.fi/stat/), the number of domestically acquired infections remained relatively stable in the study region (unpublished data). In contrast, there was an 83% increase in the consumption of chicken meat between 1997 and 2007 (https://portal.mtt.fi/portal/page/portal/mtt/mtt/julkaisut/suomenmaatalousjamaaseutuelinkeinot/jul108_SM2008.pdf). The isolates were collected from the ceca of chickens at slaughter, except during 2003, when they were isolated from retail chicken meat of three major producers from the Helsinki area during the summer months. It has been shown that the prevalence of *C. jejuni* in chicken slaughter batches (6,000 to 40,000 birds) in Finland is low in the summer; only 4% of the batches are positive at the annual level (5), and positive batches usually are 100% colonized (12). Therefore, 10 cecal samples per batch is sufficient to detect the presence of *Campylobacter* spp. in a positive batch (12). Many flocks apparently are contaminated at a late stage of rearing, and usually only one type is present at the end of the rearing period (12). Therefore, in the Finnish chicken meat chain it is probable that some strains are found both at slaughter as well as in meat. The cecal isolates from 1999 and meat isolates from 2003 were calculated to have a negative Fst value of −0.01370, which is suggestive of indistinguishable genetic populations and indicates small differences between the meat and cecal *C. jejuni* populations from 1999 and 2003.

Despite the high increase in domestically produced chicken meat consumption between 1999 and 2006, the annual overlap of STs between human and chicken isolates decreased significantly from 76% in 1999 to 58% in 2006. Also, we detected some STs either only in human isolates or only in chicken isolates. However, the Fst values between 1999 and 2006 declined from 0.06025 to 0.04602, implying that the human and chicken populations became more similar. An exception was noted in 2003, where we found an Fst value of 0.14441, which is likely the result of the fact that almost half of the STs found in 2003 in humans were novel compared to about 20% of novel STs in other years in humans. The sI_A values and genetic diversities also were calculated for each year/host separately, and generally human isolates had lower sI_A values and higher genetic diversities than those of the chicken isolates. In a study of human, poultry, and bovine isolates in Luxembourg, an Fst value of 0.01902 was calculated for the populations of human and poultry isolates (37), which is much lower than the values calculated in our study (the lowest being 0.02583). Taken together, our findings imply that not all *Campylobacter* strains found in chickens are pathogenic to humans, or that not all strains infecting humans are capable of colonizing chickens. Furthermore, the importance of chicken as a reservoir differs between countries, and our results suggest that the significance of chicken as a reservoir for domestically acquired *C. jejuni* infection has declined in the Helsinki-Uusimaa region during the study period.

The ST-45 and ST-21 CCs were the most commonly found CCs among chicken and human isolates throughout the study period and seem to be well adapted to both hosts (18, 20, 37). These CCs also have been found commonly in chicken and human isolates in the United Kingdom (3), Belgium (13), Switzerland (20), and Canada (22). The ST-45 CC in particular is highly diverse (45) and has a wide host range, including bovines, dogs, and wild birds. The ST-45 CC also has been isolated from raw milk, natural water, soil (7, 8, 21, 22, 33, 37), and even penguins in Antarctica (10). Between 2006 and 2007 we noted a decrease of 16% in the number of chicken isolates with ST-45, while the number of chicken isolates in the ST-45 CC remained stable. Both ST-3805 (9%) and ST-1326 (6%) were associated with chicken isolates and accounted for the discrepancy between the numbers of ST-45 and the ST-45 CC. ST-3805 is a novel ST identified in the present study and was found only in the chicken isolates. ST-1326 has been found previously in wild bird feces (http://pubmlst.org/campylobacter/) and, in this study, from one human isolate from 1996. Among the human isolates, no similar trend compared to that of the chicken isolates was seen; rather, relatively stable numbers of the ST-45 CC were observed. Within this CC, ST-137 and ST-230 were associated with human isolates and found only from one and two chicken isolates, respectively. Our results show that certain STs in the ST-45 CC often are detected in either humans or chickens but not in both hosts. This indicates that different sources exist for ST-45 CC-related infections in chickens and humans. Diversities calculated by both the Simpson's index of diversity and LIAN software showed that the human isolates were more diverse than the chicken isolates, suggesting that divergent genotypes come to humans from reservoirs other than chickens. A lower number (but more diverse range) of alleles as a result of clonal population expansion were found in chickens than in humans, also suggesting that reservoirs other than chickens are available to humans.

Overall, ST-50 was the most predominant ST in the ST-21 CC in Finland in both human and chicken isolates. It also was the most common ST in Belgian chicken (11) and Australian human isolates (28), whereas in the United Kingdom and Sweden ST-21 has been the major ST found in chicken and human isolates (4, 9, 41). High numbers of ST-50 were detected in humans in 2003 in this study and could indicate a common source of infection. Also, in chickens, ST-50 was found in larger amounts in 2003 than in the other studied years. Furthermore, in the human and chicken isolates from 1999, ST-883, rather than ST-50, was the major type present in the ST-21 CC. The observation that ST fluctuation in the ST-21 CC correlated between chicken and human isolates indicates that there is a common source of infection for both chickens and humans or that chickens are a major reservoir responsible for domestic acquired human infections associated with ST-50, although longitudinal studies from other potential reservoirs are needed to confirm this.

In contrast to the ST-45 and ST-21 CCs, the ST-22, ST-283, and ST-677 CCs were reported more often in Finland than in other countries (11, 20, 22, 25, 29, 37). The ST-22 CC was found only among human isolates and consisted mainly of ST-22 and ST-1947. ST-1947 was identified by Kärenlampi et al. (18) as a new ST in the ST-22 CC in human isolates from 1996, and it was the major ST in the ST-22 CC in 2006. At present, this ST has been found only in Finland and has almost completely replaced ST-22 10 years after its discovery. However, the ST-22 CC was not detected in isolates from 1999 and 2002, suggesting that its reservoirs are scarce, and humans are not always exposed to these. This could explain the strong geographical association of ST-1947 with Finland. We also

found ST-48 only among human isolates. However, in many other countries, ST-48 has been isolated frequently from both humans and chickens (20, 22, 37). This implies that different transmission routes for certain STs to humans and chickens exist between countries. These routes can be influenced by differences in weather conditions, poultry-rearing systems, contact with farm and wild animals (47), or the adaptive variation of certain genotypes, but data are scarce, and the factors underlying these differences have not been studied to date.

The ST-21, ST-45, ST-283, and ST-677 CCs were found consistently in our chicken isolates throughout the study period and were the only CCs detected in chicken isolates in 1999 and 2003. Both the ST-45 and ST-283 CCs were associated with chicken isolates, and other studies have demonstrated that both ST CCs have overlapping sources with the ST-677 complex, e.g., wild birds and environmental waters (1, 7, 8, 18, 21). Chickens and humans showed opposite trends in the prevalence of the ST-677 CC. In humans, a clear decreasing trend was evident, while in chickens an increasing trend was evident after 2003. This indicates that the chicken is a reservoir for this CC but probably does not serve as the only source of this CC in domestic human infections. In 2006, a peak in the frequency of the ST-283 CC occurred in both chickens and humans. Also, in 1999, high numbers of the ST-283 CC were found in humans. This indicates that the chicken is a reservoir of this ST for domestic human infections, but other reservoirs also may exist.

In conclusion, we have shown that over the course of a decade different STs fluctuated from year to year in both patient and chicken isolates. These fluctuations in human and chicken isolates seemed not to be directly linked in the predominant STs. Furthermore, the overlap between STs in the two hosts constantly decreased. Thus, the role of the chicken as a reservoir for domestically acquired human infections in Finland apparently has decreased despite the simultaneous elevation in the consumption of chicken meat. In addition, the greater genetic diversity of human isolates suggests a range of reservoirs for domestically acquired human campylobacteriosis cases.

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