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Temporal and Geographical Distribution and Overlap of Penner Heat-Stable Serotypes and Pulsed-Field Gel Electrophoresis Genotypes of *Campylobacter jejuni* Isolates Collected from Humans and Chickens in Finland during a Seasonal Peak

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The association of Penner heat-stable serotypes and pulsed-field gel electrophoresis genotypes of 208 human and 30 chicken *Campylobacter jejuni* isolates was studied. Overall, 46% of the human strains had overlapping sero- and genotype combinations with chicken strains. The percentage was reduced to 31% for strains that were considered temporally related. This suggests common environmental sources.

Campylobacter jejuni is the most frequent bacterial cause of human gastroenteritis in developed countries worldwide (3, 11). Case control studies have indicated that improper handling of poultry products and eating raw or undercooked chicken are important risk factors for human campylobacter infections. Yet, most campylobacter infections are sporadic, and the sources of infection remain unidentified. Human campylobacter infections in northern Europe show a peak during the summer months of July, August, and September (9). In Finland, most domestic campylobacter infections occur from July to August, and the number of campylobacter-positive chicken flocks increases during the same time period (6, 11).

Serotyping has traditionally been used for typing *C. jejuni* isolates, but pulsed-field gel electrophoresis (PFGE) using *Sma*I and *Sac*II/*Kpn*I has been identified as a more highly discriminatory method of studying the epidemiology of *C. jejuni* infection (4, 6, 7). Overlapping serotypes and PFGE genotypes have been identified in chicken and human isolates (2, 6, 8), but the geographical and temporal overlap of such strains has not been studied extensively.

In an earlier study (15), all domestic-laboratory-confirmed human *C. jejuni* isolates (533) from sporadic cases were collected from the whole of Finland from July to September 1999. Thirty chicken *C. jejuni* isolates were collected during the same seasonal peak from every positive flock at three major abattoirs, accounting for 98% of chicken meat production in Finland (10). In the present study 208, previously serotyped, human *C. jejuni* strains were typed by PFGE and their association with the chicken strains was evaluated. The human strains originated from six hospital districts in Finland (271 isolates in total) and included 10 Penner heat-stable (HS) serotypes, also identified in chicken flocks during the same seasonal peak (HS1/44, HS2, HS4 complex, HS5, HS6/7, HS11, HS12, HS27, HS41, and HS57), as well as nonserotypeable (NS) human strains.

The DNA plugs for PFGE analysis were prepared as previously described (5, 7, 12). The DNA was digested by *Sma*I and *Kpn*I (New England Biolabs Inc.; 20 U per sample), and the restriction fragments were separated with ramped pulses of 1 to 30 s and 1 to 25 s for 19 h, respectively. After computer-assisted (BioNumerics, version 3.0; Applied Maths, Kortrijk, Belgium) and visual analyses of the patterns, clusters of closely related and indistinguishable genotypes were identified. PFGE genotypes were considered closely related if they differed by one to three bands and were considered indistinguishable if they had all bands in common (14). The combined *Sma*I/*Kpn*I genotypes were named C1, C2, etc.

A restriction pattern with *Kpn*I was not obtained for 4 of the 208 human strains (2%). The DNA of 44 (18%) strains was not digested by *Sma*I, and 39 of these strains (83%) were of serotype HS6/7. Overall *Sma*I digestion yielded 48, *Kpn*I digestion yielded 74, and the combination yielded 77 PFGE genotypes. The strains formed 22 clusters of indistinguishable or closely related *Sma*I/*Kpn*I PFGE genotypes. Eight of the clusters consisted of only human strains.

The predominant human sero- and genotype combinations (sero/genotypes) were dispersed across the studied districts all over Finland. Most of the predominant human genotypes were also identified in chickens. However, two common and closely related genotypes within the serotype HS4 complex and two predominant closely related genotypes within serotype HS27 were not isolated from chickens. Similarly, a genotype associated with serotype HS1/44 was isolated from three separate districts, but not from chickens. Two geographically and temporally associated clusters of human strains were identified in the northern district, one associated with serotype HS2 (four

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TABLE 1. Temporal association of Penner heat-stable serotypes and PFGE genotypes[a] of chicken and human *C. jejuni* isolates during a seasonal peak

Date of sampling (day.mo.yr)	Serotype/ genotype[c]	No. of human isolates after sampling a positive chicken flock			
		<2 days	2–23 days	>23 days	Total
17.6.99	6/7/C3	NC[d]	3	0	3
22.6.99	27/C4	NC	9	9	18
7.7.99	12/C1	2	6 + 5[b]	15	28
8.7.99	NS/C5	0	2	2	4
9.7.99	6/7/C3	3	0	0	
12.7.99	6/7/C3	3	0	0	
12.7.99	6/7/C3	3	0	0	
12.7.99	6/7/C9	4	27	5	36
13.7.99	6/7/C3	3	0	0	
15.7.99	12/C1	8	3 + 5[b]	12	
16.7.99	4 complex/C7	3	0	1	4
19.7.99	4 complex/C8	2	0	0	2
19.7.99	1/44/C4	0	0	0	0
20.7.99	41/C2	2	1	0	3
20.7.99	57/C15	4	2	1	7
26.7.99	12/C10	1	5[b]	0	6
28.7.99	NS/C4	0	0	0	0
29.7.99	12/C10	1	5[b]	0	
2.8.99	NS/C5	2	0	2	
9.8.99	6/7/C9	33	2[b]	1	
9.8.99	6/7/C9	33	2[b]	1	
9.8.99	27/C4	16	2	0	
16.8.99	NS/C16	0	2	0	2
16.8.99	2/C11	0	9	2	11
23.8.99	1/44/C12	0	0	0	0
23.8.99	12/C1	20	5	3	
16.9.99	5/C13	1	0	0	1
20.9.99	NS/C19	0	0	0	0
22.9.99	11/C14	0	0	0	0
27.9.99	NS/C17	0	0	0	0

[a] Indistinguishable and closely related genotypes as defined previously (14).
[b] The numbers overlap because the time of chicken sampling at slaughter could not differentiate which human isolates might have been associated with the indistinguishable sero- and genotype combinations found in chickens.
[c] All serotypes have the prefix HS omitted.
[d] NC, not collected.

strains) and the other with the HS4 complex (three strains). Genotypes occurring in September differed from those seen in July and August and were more often unique. Twenty-eight strains, 3 of which were from chickens, had unique PFGE genotypes.

Overall, 46% of all the laboratory-confirmed human isolates from the six studied hospital districts had overlapping sero- and genotype combinations with those found in chickens (34% indistinguishable). For these strains the temporal association of the date of chicken fecal sampling at slaughter and that of human fecal sampling for *C. jejuni* was evaluated (Table 1). The dates were considered temporally related when patients were sampled 2 to 23 days after the positive chicken flock. The time criteria were chosen assuming that the chicken products reached market the day after slaughter, that the "use by" date indicated on the packages was 10 days after slaughter, that the incubation time of the infection was 1 to 7 days, and that human fecal sampling was carried out at the latest 6 days after the first symptoms appeared. The percentage of overlapping sero- and genotype combinations was reduced to 31% (21% indistinguishable) for temporally related human and chicken strains. When the same sero- and genotype combination was identified from several chicken flocks at slaughter, it was not possible to evaluate the temporal association in more detail. Thirteen sero- and genotype combinations were associated with only one chicken flock and clustered with those of 30 (11%) human strains. Only 14 (47%) of the 30 strains were isolated 2 to 23 days (average, 14 days) after the positive flock was slaughtered. Twelve and 4 strains were isolated either an average of 9 days before or over 23 days after (average, 30 days), respectively, the positive chicken flock was slaughtered.

The transfer of a particular genotype through Finland provided some additional evidence on the importance of environmental sources in *C. jejuni* infections. The most common genotype (C7) associated with the serotype HS4 complex (five strains) and NS strains (six strains) was first isolated in the southern district in the beginning of July, followed by the southwestern, western, and eastern districts in the middle of July and finally the northern district at the end of July and in August. It was also identified in a chicken flock before identification in the northern district, suggesting that some environmental vectors may have transferred the *C. jejuni* strains from one area to another. It has been suggested that wild birds are possible reservoirs or vectors for human *C. jejuni* infections (1, 13).

In conclusion, 34% of the sporadic *C. jejuni* infections during the seasonal peak in 1999 in Finland were caused by indistinguishable sero- and genotype combinations found in chicken flocks at slaughter, suggesting that chickens may be a source of human infections, either directly or by increasing the environmental load of *C. jejuni*. However, human strains with overlapping sero- and genotype combinations with a chicken strain were also isolated prior to the slaughter of the chicken flock, suggesting common environmental sources for both human infection and flock contamination during the seasonal peak. Other vehicles distributing the strains throughout the country remain to be identified.

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