Evaluation of Genetic Markers and Molecular Typing Methods for Prediction of Sources of *Campylobacter jejuni* and *C. coli* Infections[▽]

Rauni Kärenlampi,[1]* Hilpi Rautelin,[2] and Marja-Liisa Hänninen[1]

*Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland,[1] and
Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, and HUSLAB,
Helsinki University Central Hospital Laboratory, Helsinki, Finland[2]*

***Campylobacter jejuni* and *C. coli* isolates from poultry, cattle, and humans were studied using pulsed-field gel electrophoresis (PFGE) and PCR of candidate livestock-associated marker genes. Human isolates showed 5.7 and 61% overlap with cattle and poultry isolates, respectively, by use of PFGE. No unambiguous association was found between marker genes (the Cj1321 and Cj1324 genes) and livestock-associated isolates.**

Campylobacter jejuni and *C. coli* constitute the leading cause of bacterial gastroenteritis in Finland (www.ktl.fi/ttr) (13), and tasting or eating raw or undercooked meat, drinking water from a dug well, and swimming in natural sources of water have been reported as the most important risk factors for domestically acquired sporadic infections (15), which show a clear seasonal peak, especially in July and August (8, 13). The relative contributions of different sources of infection are not well known. Molecular typing methods, including pulsed-field gel electrophoresis (PFGE), targeted at the identification of possible reservoirs and sources of infection have been widely utilized to study the molecular epidemiology of *Campylobacter* infections. More recently, a multilocus sequence typing (MLST) method has been described for the study of the population biology of *C. jejuni* and *C. coli* (3, 4). PFGE using SmaI restriction digestion has been shown to be more discriminatory than MLST for outbreak investigations (14). In addition, PFGE utilizing KpnI restriction digestion has been shown to be more discriminatory than SmaI typing for comparison of *C. jejuni* isolates (7, 11). Recently, a cluster of six genes (the Cj1321 gene to the Cj1326 gene) within the O-linked flagellin glycosylation locus was proposed to be a suitable genetic marker predictive of sources of *C. jejuni* infections based on comparative microarray analysis of strains isolated from humans, chickens, bovines, ovines, and the environment (1).

The aims of our study were (i) to evaluate the discriminatory power of PFGE with KpnI restriction digestion for typing Finnish *C. jejuni* and *C. coli* isolates previously characterized by MLST (8); (ii) to study the overlap of PFGE profiles of isolates from human domestically acquired sporadic infections from the Helsinki-Uusimaa area with those of isolates from cattle fecal and chicken retail meat samples during the same seasonal peak, from July to September 2003, in Finland; and (iii) to study the association of two genetic markers, the Cj1321 and Cj1324 genes, which have been suggested to be livestock-associated *C. jejuni* markers predictive of infection source (1), with the source of isolation.

Altogether, 70 human, 32 chicken and 4 turkey retail meat, and 20 cattle fecal isolates were collected during the seasonal peak from July to September in 2003 and were typed by MLST as previously reported (8). All human strains were non-outbreak related and were isolated from patients who had acquired their infections from domestic sources and were diagnosed at the Helsinki University Central Hospital Laboratory. During the same seasonal peak, poultry isolates were collected from the Helsinki area and cattle fecal samples were collected from different Finnish slaughterhouses all over the country.

PFGE was performed as described previously (6, 10) using KpnI (New England Biolabs Inc.; 20 U per sample) restriction digestion. PFGE data were analyzed with BioNumerics V. 4.01 software (Applied Maths, Kortrijk, Belgium) using the Dice similarity coefficient, with 0.5% optimization and 1% tolerance. Clustering was done with the unweighted pair group method using arithmetic averages. New PFGE KpnI profiles were assigned for each pattern differing by at least one band.

PCR primers and reactions for the amplification of the Cj1321 and Cj1324 loci were as described by Champion et al. (1). The cycling conditions were as follows: denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min for 35 cycles in total. Strain NCTC11168 was used as a positive control for the PCRs.

The distribution of MLST sequence types (STs) and PFGE and PCR profiles of human, cattle, and poultry isolates are shown in Fig. 1. In most cases, PFGE analysis further discriminated between isolates among MLST STs. This was especially pronounced among ST-45 isolates. PFGE analysis using KpnI resulted in 5.7 and 61% overlap between human and cattle isolates and human and poultry isolates, respectively. This is in line with the data reported in the EU showing that 3.3 and 30% of the outbreaks in 2004 were attributable to bovine and poultry meat, respectively (5), and with numerous case-control studies in which poultry meat has been identified as a major risk factor for sporadic cases of campylobacteriosis (13). However, more-accurate estimations for source attribution of the sporadic domestically acquired cases could have been obtained by comparing larger numbers of isolates from humans with those from both types of meat at the retail level, as all strains

* Corresponding author. Mailing address: Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, P.O. Box 66, FI-00014 University of Helsinki, Finland. Phone: 358 9 191 57117. Fax: 358 9 191 57101. E-mail: rauni.karenlampi@helsinki.fi.

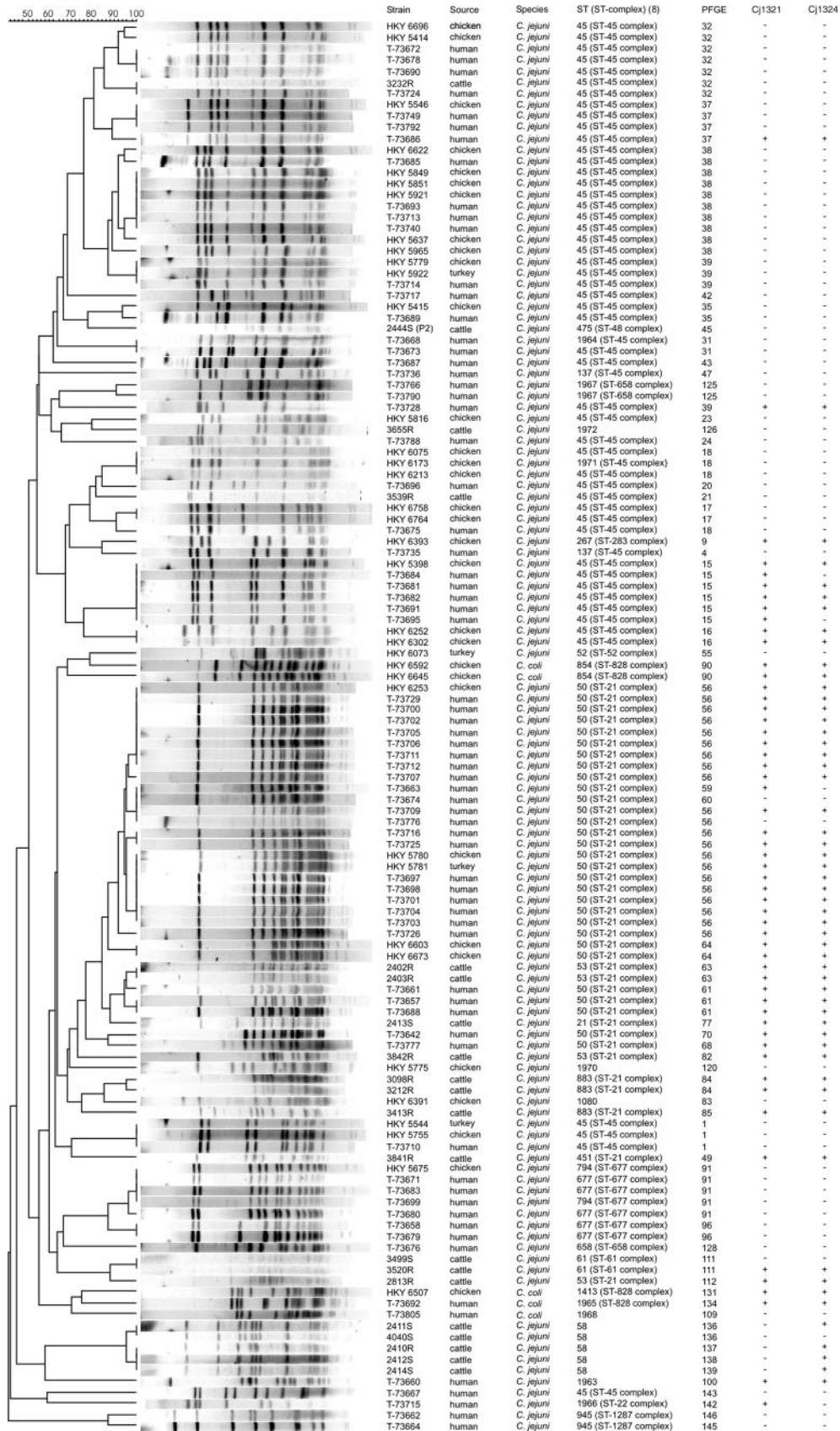| Strain	Source	Species	ST (ST-complex) (8)	PFGE	Cj1321	Cj1324
HKY 6696	chicken	C. jejuni	45 (ST-45 complex)	32	-	-
HKY 5414	chicken	C. jejuni	45 (ST-45 complex)	32	-	-
T-73672	human	C. jejuni	45 (ST-45 complex)	32	-	-
T-73678	human	C. jejuni	45 (ST-45 complex)	32	-	-
T-73690	human	C. jejuni	45 (ST-45 complex)	32	-	-
3232R	cattle	C. jejuni	45 (ST-45 complex)	32	-	-
T-73724	human	C. jejuni	45 (ST-45 complex)	32	-	-
HKY 5546	chicken	C. jejuni	45 (ST-45 complex)	37	-	-
T-73749	human	C. jejuni	45 (ST-45 complex)	37	-	-
T-73792	human	C. jejuni	45 (ST-45 complex)	37	-	-
T-73686	human	C. jejuni	45 (ST-45 complex)	37	+	+
HKY 6622	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
T-73685	human	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5849	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5851	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5921	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
T-73693	human	C. jejuni	45 (ST-45 complex)	38	-	-
T-73713	human	C. jejuni	45 (ST-45 complex)	38	-	-
T-73740	human	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5637	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5965	chicken	C. jejuni	45 (ST-45 complex)	38	-	-
HKY 5779	chicken	C. jejuni	45 (ST-45 complex)	39	-	-
HKY 5922	turkey	C. jejuni	45 (ST-45 complex)	39	-	-
T-73714	human	C. jejuni	45 (ST-45 complex)	39	-	-
T-73717	human	C. jejuni	45 (ST-45 complex)	42	-	-
HKY 5415	chicken	C. jejuni	45 (ST-45 complex)	35	-	-
T-73689	human	C. jejuni	45 (ST-45 complex)	35	-	-
2444S (P2)	cattle	C. jejuni	475 (ST-48 complex)	45	-	-
T-73668	human	C. jejuni	1964 (ST-45 complex)	31	-	-
T-73673	human	C. jejuni	45 (ST-45 complex)	31	-	-
T-73687	human	C. jejuni	45 (ST-45 complex)	43	-	-
T-73736	human	C. jejuni	137 (ST-45 complex)	47	-	-
T-73766	human	C. jejuni	1967 (ST-658 complex)	125	-	-
T-73790	human	C. jejuni	1967 (ST-658 complex)	125	-	-
T-73728	human	C. jejuni	45 (ST-45 complex)	39	+	+
HKY 5816	chicken	C. jejuni	45 (ST-45 complex)	23	-	-
3655R	cattle	C. jejuni	1972	126	-	-
T-73788	human	C. jejuni	45 (ST-45 complex)	24	-	-
HKY 6075	chicken	C. jejuni	45 (ST-45 complex)	18	-	-
HKY 6173	chicken	C. jejuni	1971 (ST-45 complex)	18	-	-
HKY 6213	chicken	C. jejuni	45 (ST-45 complex)	18	-	-
T-73696	human	C. jejuni	45 (ST-45 complex)	20	-	-
3539R	cattle	C. jejuni	45 (ST-45 complex)	21	-	-
HKY 6758	chicken	C. jejuni	45 (ST-45 complex)	17	-	-
HKY 6764	chicken	C. jejuni	45 (ST-45 complex)	17	-	-
T-73675	human	C. jejuni	45 (ST-45 complex)	18	-	-
HKY 6393	chicken	C. jejuni	267 (ST-283 complex)	9	+	+
T-73735	human	C. jejuni	137 (ST-45 complex)	4	-	-
HKY 5398	chicken	C. jejuni	45 (ST-45 complex)	15	+	+
T-73684	human	C. jejuni	45 (ST-45 complex)	15	+	+
T-73681	human	C. jejuni	45 (ST-45 complex)	15	+	+
T-73682	human	C. jejuni	45 (ST-45 complex)	15	+	+
T-73691	human	C. jejuni	45 (ST-45 complex)	15	+	+
T-73695	human	C. jejuni	45 (ST-45 complex)	15	+	-
HKY 6252	chicken	C. jejuni	45 (ST-45 complex)	16	+	+
HKY 6302	chicken	C. jejuni	45 (ST-45 complex)	16	+	+
HKY 6073	turkey	C. jejuni	52 (ST-52 complex)	55	+	+
HKY 6592	chicken	C. coli	854 (ST-828 complex)	90	+	+
HKY 6645	chicken	C. coli	854 (ST-828 complex)	90	+	+
HKY 6253	chicken	C. jejuni	50 (ST-21 complex)	56	+	+
T-73729	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73700	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73702	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73705	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73706	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73711	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73712	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73707	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73663	human	C. jejuni	50 (ST-21 complex)	59	+	-
T-73674	human	C. jejuni	50 (ST-21 complex)	60	-	-
T-73709	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73776	human	C. jejuni	50 (ST-21 complex)	56	-	-
T-73716	human	C. jejuni	50 (ST-21 complex)	56	-	-
T-73725	human	C. jejuni	50 (ST-21 complex)	56	+	+
HKY 5780	chicken	C. jejuni	50 (ST-21 complex)	56	+	+
HKY 5781	turkey	C. jejuni	50 (ST-21 complex)	56	+	+
T-73697	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73698	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73701	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73704	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73703	human	C. jejuni	50 (ST-21 complex)	56	+	+
T-73726	human	C. jejuni	50 (ST-21 complex)	56	+	+
HKY 6603	chicken	C. jejuni	50 (ST-21 complex)	64	+	+
HKY 6673	chicken	C. jejuni	50 (ST-21 complex)	64	+	+
2402R	cattle	C. jejuni	53 (ST-21 complex)	63	+	+
2403R	cattle	C. jejuni	53 (ST-21 complex)	63	+	+
T-73661	human	C. jejuni	50 (ST-21 complex)	61	+	+
T-73657	human	C. jejuni	50 (ST-21 complex)	61	+	+
T-73688	human	C. jejuni	50 (ST-21 complex)	61	+	+
2413S	cattle	C. jejuni	21 (ST-21 complex)	77	+	+
T-73642	human	C. jejuni	50 (ST-21 complex)	70	+	+
T-73777	human	C. jejuni	50 (ST-21 complex)	68	+	+
3842R	cattle	C. jejuni	53 (ST-21 complex)	82	+	+
HKY 5775	chicken	C. jejuni	1970	120	+	+
3098R	cattle	C. jejuni	883 (ST-21 complex)	84	+	+
3212R	cattle	C. jejuni	883 (ST-21 complex)	84	+	+
HKY 6391	chicken	C. jejuni	1080	83	+	+
3413R	cattle	C. jejuni	883 (ST-21 complex)	85	+	+
HKY 5544	turkey	C. jejuni	45 (ST-45 complex)	1	-	-
HKY 5755	chicken	C. jejuni	45 (ST-45 complex)	1	-	-
T-73710	human	C. jejuni	45 (ST-45 complex)	1	-	-
3841R	cattle	C. jejuni	451 (ST-21 complex)	49	+	+
HKY 5675	chicken	C. jejuni	794 (ST-677 complex)	91	-	-
T-73671	human	C. jejuni	677 (ST-677 complex)	91	-	-
T-73683	human	C. jejuni	677 (ST-677 complex)	91	-	-
T-73699	human	C. jejuni	794 (ST-677 complex)	91	-	-
T-73680	human	C. jejuni	677 (ST-677 complex)	91	-	-
T-73658	human	C. jejuni	677 (ST-677 complex)	96	-	-
T-73679	human	C. jejuni	677 (ST-677 complex)	96	-	-
T-73676	human	C. jejuni	658 (ST-658 complex)	128	-	-
3499S	cattle	C. jejuni	61 (ST-61 complex)	111	-	-
3520R	cattle	C. jejuni	61 (ST-61 complex)	111	+	+
2813R	cattle	C. jejuni	53 (ST-21 complex)	112	+	+
HKY 6507	chicken	C. coli	1413 (ST-828 complex)	131	+	+
T-73692	human	C. coli	1965 (ST-828 complex)	134	+	+
T-73805	human	C. coli	1968	109	-	+
2411S	cattle	C. jejuni	58	136	-	+
4040S	cattle	C. jejuni	58	136	-	+
2410R	cattle	C. jejuni	58	137	-	+
2412S	cattle	C. jejuni	58	138	-	+
2414S	cattle	C. jejuni	58	139	-	+
T-73660	human	C. jejuni	1963	100	+	+
T-73667	human	C. jejuni	45 (ST-45 complex)	143	-	-
T-73715	human	C. jejuni	1966 (ST-22 complex)	142	+	-
T-73662	human	C. jejuni	945 (ST-1287 complex)	146	-	-
T-73664	human	C. jejuni	945 (ST-1287 complex)	145	-	-

FIG. 1. Results for PFGE using KpnI restriction enzyme and PCR for two genetic markers the Cj1321 and Cj1324 genes (1), from typing Finnish human, cattle, and poultry isolates of C. jejuni and C. coli collected during the seasonal peak from July to September in 2003.

may not survive to be found on the final products (12), and by considering the temporal association of the isolates (7).

Overall, a large percentage of the poultry and cattle isolates were shown to be PCR negative for the Cj1321 and Cj1324 genes (Fig. 1). The percentages of isolates positive for the genes by PCR among human, cattle, chicken, and turkey isolates were 47, 50, 34 and 25%, respectively, for the Cj1321 gene and 41, 70, 34 and 25%, respectively, for the Cj1324 gene. The ST-45 complex, previously found to be overrepresented among poultry isolates (2, 8, 9), was significantly associated with the absence of both genes ($P < 0.05$; χ^2 test). In most cases, the PCR results for both genetic loci were concordant (i.e., either positive or negative). However, cattle isolates were significantly associated with the Cj1324 gene. Our findings diverged from those reported by Champion et al. (1), who suggested the Cj1321-Cj1326 gene cluster to be livestock associated based on microarray analysis of 70 *C. jejuni* strains isolated from humans, 17 from chickens, 13 from bovines, 5 from ovines, and 6 from beaches. This may reflect differences between the *C. jejuni* populations in livestock in Finland and those in the United Kingdom; however, our results stress the need to confirm the results obtained by microarray comparative genomic hybridization using a variety of *C. jejuni* strains from geographically diverse origins.

In conclusion, MLST and especially the more discriminatory PFGE analysis using the KpnI restriction enzyme suggested a role for poultry more important than that for cattle as a reservoir of *Campylobacter* subtypes identified in domestically acquired sporadic infections during the seasonal peak in 2003 in Finland. In addition, our study concerning a variety of *Campylobacter* isolates from Finland did not show a clear relationship between livestock-associated isolates and the previously (1) proposed Cj1321 and Cj1324 genetic markers.

REFERENCES

1. **Champion, O. L., M. W. Gaunt, O. Gundogdu, A. Elmi, A. A. Witney, J. Hinds, N. Dorrell, and B. W. Wren.** 2005. Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. Proc. Natl. Acad. Sci. USA **102:**16043–16048.
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. **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.
4. **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.
5. **European Food Safety Authority.** 2005. The community summary report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004. EFSA J. **310:**1–278.
6. **Hänninen, M.-L., P. Perko-Mäkelä, A. Pitkälä, and H. Rautelin.** 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin. Microbiol. **38:**1998–2000.
7. **Kärenlampi, R., H. Rautelin, M. Hakkinen, and M.-L. Hänninen.** 2003. 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. J. Clin. Microbiol. **41:**4870–4872.
8. **Kärenlampi, R., H. Rautelin, D. Schönberg-Norio, L. Paulin, and M.-L. Hänninen.** 2007. 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. Appl. Environ. Microbiol. **73:**148–155.
9. **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.
10. **Maslow, J. N., A. M. Slutsky, and R. D. Arbeit.** 1993. Application of pulsed-field gel electrophoresis to molecular epidemiology, p. 563–572. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. ASM Press, Washington, DC.
11. **Michaud, S., S. Menard, C. Gaudreau, and R. D. Arbeit.** 2001. Comparison of SmaI-defined genotypes of *Campylobacter jejuni* examined by KpnI: a population-based study. J. Med. Microbiol. **50:**1075–1081.
12. **Newell, D. G., J. E. Shreeve, M. Toszeghy, G. Domingue, S. Bull, T. Humphrey, and G. Mead.** 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. Appl. Environ. Microbiol. **67:**2636–2640.
13. **Rautelin, H., and M.-L. Hänninen.** 2000. Campylobacters: the most common bacterial enteropathogens in the Nordic countries. Ann. Med. **32:**440–445.
14. **Sails, A. D., B. Swaminathan, and P. I. Fields.** 2003. Utility of multilocus sequence typing as an epidemiological tool for investigation of outbreaks of gastroenteritis caused by *Campylobacter jejuni*. J. Clin. Microbiol. **41:**4733–4739.
15. **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.