

## Prevalence of campylobacters in chicken flocks during the summer of 1999 in Finland

P. PERKO-MÄKELÄ<sup>1</sup>\*, M. HAKKINEN<sup>2</sup>, T. HONKANEN-BUZALSKI<sup>2</sup>  
AND M.-L. HÄNNINEN<sup>1</sup>

<sup>1</sup> Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, P.O. Box 57, 00014 University of Helsinki, Finland

<sup>2</sup> National Veterinary and Food Research Institute, P.O. Box 45, 00581 Helsinki, Finland

(Accepted 3 April 2002)

### SUMMARY

In order to determine the prevalence of campylobacter positive broiler flocks in Finland, every flock from all three major slaughterhouses was studied during the period from 1 May to 30 September 1999. Caecal samples were taken in the slaughterhouses from five birds per flock. A total of 1132 broiler flocks were tested and 33 (2.9%) of those were campylobacter positive. Thirty-one isolates were *C. jejuni* and two isolates were *C. coli*. Isolates were serotyped for heat-stable antigens (Penner) and genotyped with pulsed-field gel electrophoresis (PFGE). The most common serotypes were serotypes 6, 7, 12 and 4-complex. Together with *SmaI* and *KpnI* patterns there were 18 different PFGE genotypes. Simultaneous monitoring of chicken flocks and typing of the isolates produced data which can be used to study the epidemiology of campylobacters in chicken as well as their role in human infections.

### INTRODUCTION

In Finland, as in many Western European countries the number of reported human campylobacter cases has increased during recent years. Latterly the number of campylobacter cases has exceeded that of the reported number of salmonella cases [1, 2]. In 1999, 3303 campylobacter and 2801 salmonella infections were reported in Finland [2]. In epidemiological studies handling or eating poultry have been shown to be significant risk factors for human infections [3, 4]. The contamination rate of poultry at the retail level varies between different countries from 14–98% [5]. In Finland, the contamination rate at retail markets in the Helsinki area during the seasonal peak in July to August in 1996–9 was 10–30% [6].

Decreasing the prevalence of campylobacter colonized broiler flocks is considered to be one of the most effective ways to reduce the number of campylobacter positive poultry products [7, 8]. This ensures the

microbiological safety of fresh chicken for human consumption. Although slaughtering technique and processing hygiene have improved, the contamination of carcasses from intestinal contents is not likely to be completely prevented [9, 10].

Prevalence studies on campylobacter positive poultry flocks in Europe have been made and results vary from 18% in Norway to 82% in The Netherlands [11]. In many studies a seasonal variation of the prevalence of campylobacter colonized flocks has been seen. Higher recovery rates have been detected during the summer months, June, July and August, compared to winter [7, 8, 12].

There are only limited data on the prevalence of campylobacters in chicken flocks in Finland. In 1988 Aho and Hirn [13] published a study in which they reported that 24% (117/490) of caecal samples at slaughter were campylobacter positive. The three major slaughterhouses do their own-check studies but there has not been any permanent monitoring programme controlled by authority in which all slaughter-

\* Author for correspondence.

houses have participated. In 1999, from 1 May to 30 September, every flock from all three major slaughterhouses which account for 98% of Finnish chicken meat production were studied in order to determine the prevalence of campylobacter positive broiler flocks. In order to get more data on diversity of campylobacter isolates and to compare similarity of chicken and human isolates for epidemiological purposes, serotyping with heat stable antigens and genotyping with pulsed-field gel electrophoresis was performed. This study was a co-operation between the slaughterhouses, the National Veterinary and Food Research Institute and the Department of Food and Environmental Hygiene.

## MATERIALS AND METHODS

### Study design

The study population consisted of 1132 broiler flocks which included all the slaughtered flocks of the three major Finnish poultry companies from 1 May to 30 September in 1999. These three companies produce approximately 98% of the broiler meat produced in Finland. Broiler chickens are slaughtered at the age of 35–42 days and the entire flock is slaughtered on the same day or on 2 subsequent days.

### Sampling

Caecal samples were taken from slaughterhouses by sampling of five birds from each flock. The size of the flock varied from 3500–45000 birds, the most usual flock size being 15000 or 30000 birds. The number of studied caecal samples, five, was estimated to detect campylobacter positive flocks at a confidence level of 97.5% from population size up to 45000 birds with an estimated prevalence of 60% within the infected flock. If at least one of the five samples was positive the flock was classified as positive.

Caecal samples were taken by broiler-company personnel at the point of meat inspection of viscera. Individual samples were cultured in the laboratories of the participating slaughterhouses.

### Bacteriological methods

Caecal samples were analysed for campylobacter using a modified version of the procedure described by Bolton et al. [14] for isolation of campylobacter from faeces. Caecal contents were cultured by direct

plating on modified CCD agar (Oxoid Ltd., Basingstoke, Hampshire, UK). The plates were incubated in a microaerobic atmosphere at 42 °C for 48 h. Two typical colonies were subcultured and sent for further analysis to the National Veterinary and Food Research Institute and the Department of Food and Environmental Hygiene.

Isolates were identified to the species level by the use of Gram-staining, phase contrast microscopy for motility, oxidase, catalase, hippurate hydrolysis and susceptibility to nalidixic acid (30 µg/ml) according to a modified procedure of the Nordic Committee on Food Analysis [15]. One isolate from each positive flock was taken for sero- and genotyping studies.

### Serotyping

*Campylobacter jejuni* isolates were serotyped with a commercial reagent for the serotyping of heat stable antigens (Penner) of campylobacters by the passive haemagglutination method (Denka Seiken Co., Ltd. Tokyo, Japan).

### Genotyping with pulsed-field gel electrophoresis

For genotyping with PFGE all isolates were cultured on brucella blood agar (Oxoid) plates incubated at 37 °C in a microaerobic atmosphere for 24–40 h. The bacterial cells were harvested and DNA plugs were prepared as described earlier [16, 17]. The DNA plug slices were digested with *Sma*I or *Kpn*I restriction enzymes (New England Biolabs, Hertfordshire, UK) as described by the manufacturer. *Sma*I and *Kpn*I fragments were separated with a ramped pulse of 0.5–40 sec for 19 h or 1–25 sec for 20 h, respectively. Otherwise, electrophoresis conditions were as described earlier [16].

If the isolates had one or more differences in *Sma*I bands they were considered as different patterns and named as S1, S2 and so on. If they had five or more different bands in *Kpn*I they were considered as differing patterns and named as K1, K2 and so on. Together these two patterns were combined and named as genotype C1, C2 and so on. The schema has been used in our earlier studies [16].

## RESULTS

The overall campylobacter-positive flock prevalence was 2.9% (33 of the total 1132 flocks studied) during the period from 1 May to 30 September 1999. The

Table 1. *Campylobacter* positive farms and characterization of campylobacter isolates by sero- and genotyping

Isolate	Month of isolation	Farm	No. of birds in the flock	No. of campylobacter positive houses/ total no. of houses	Serotype (Penner)	PFGE genotype		
						<i>Sma</i> I pattern	<i>Kpn</i> I pattern	Combined
1831	May	A	15 500	1/2	4, 13, 16, 43, 50	S3	K6	C6
1959	June	B	37 500	1/1	6, 7	S2	K3	C3
2059	June	C	15 000	1/1	27	S2	K4	C4
2165	July	D	30 000	1/1	12	S1	K1	C1
2166	July	E	15 000	1/2	NS	S2	K5	C5
2186	July	B	3 000	4/4	6, 7	S2	K3	C3
2197	July	B	7 000		6, 7	S2	K3	C3
2199	July	B	15 000		ND	S2	K3	C3
2213	July	B	10 000		6, 7	S2	K3	C3
2219	July	F	12 500	1/1	4, 13, 16, 43, 50	S4	K6	C7
2227	July	G	30 000	1/1	6, 7	UD	K8	C9
2230	July	H	30 000	1/1	12	S1	K1	C1
2232	July	I	11 000	2/2	4, 13, 16, 43, 50	S5	K7	C8
2234	July	I	11 000		ND	S2	K4	C4
2252	July	J	7 000	1/1	41	S1	K2	C2
2347	July	K	15 000	1/1	57	S11	K13	C15
2351	July	L	30 000	1/2	12	S6	K9	C10
2360	July	M	30 000	1/1	NS	S2	K5	C5
2361	July	N	30 000	1/2	12	S6	K9	C10
2362	Aug.	H	30 000	1/1	NS	S2	K4	C4
2447	Aug.	O	15 000	2/2	<i>C. coli</i>	S14	K15	C18
2448	Aug.	O	15 000		<i>C. coli</i>	S14	K15	C18
2449	Aug.	P	15 000	1/1	6, 7	UD	K8	C9
2450	Aug.	Q	30 000	1/2	6, 7	UD	K8	C9
2458	Aug.	B	44 000	1/1	27	S2	K4	C4
2538	Aug.	R	30 000	2/2	NS	S12	K13	C16
2539	Aug.	R	30 000		ND	S7	K10	C11
2541	Aug.	S	30 000	2/2	ND	S8	K11	C12
2542	Aug.	S	30 000		ND	S1	K1	C1
2867	Sept.	J	7 000	1/1	5	S9	K12	C13
2899	Sept.	T	27 000	1/2	NS	—	—	—
2946	Sept.	U	8 000	1/5	11	S10	K12	C14
2965	Sept.	V	15 500	1/3	NS	S13	K14	C17

NS, nonserotypable; ND, not done; UD, undigested.

sizes of campylobacter positive flocks varied: < 10 000 (5), 10 000–15 500 (11), 15 600–20 000 (0), 20 000–30 000 (12), and > 30 000 (2) (Table 1). During this time approximately 19 700 000 broilers were slaughtered and 606 000 (3%) of these were campylobacter positive, if the whole flock was assumed to be positive when one sample was positive. The monthly variation in the number of campylobacter positive flocks is shown in Table 2. Thirty-one of the isolates were *C. jejuni* (94%) and two were *C. coli* (6%). Positive flocks were from 22 farms. The total number of studied farms was 220. Three of the farms had positive flocks subsequently (B, H and J, Table 1). Thirteen of the positive farms had two or more broiler houses. In

eight of these farms only one of the houses was positive for campylobacter. Five farms that had several houses had every house positive for campylobacter (Table 1).

### Serotypes

Eight serotypes were identified among 26 isolates which were serotyped (Table 2). Six of the isolates were nonserotypable with the available set of sero-specific sera. Serotype 6, 7 was the most common serotype found (7/26) and serotypes 12, 4-complex and 27 were isolated more than once. Serotypes 6, 7 and 27 were found in June, July and August. Serotype

Table 2. Monthly variation in the number of campylobacter positive flocks

Month	No. of flocks	No. of positive flocks	%
May	227	1	0.4
June	224	2	0.9
July	230	16	7.0
Aug.	220	10	4.5
Sept.	231	4	1.7
Total	1132	33	2.9

12 was seen in July and August. The 4-complex serotype was seen in May and July. In September unique serotypes 5 and 11 were found. Serotypes of subsequent campylobacter positive flocks at farm B were 6, 7 in June and 27 in August, at farm J, 41 in July and 5 in September, and at farm H, 12 in July and nonserotypable in August (Table 2). Serotypes 6, 7, 27 and 4-complex were identified in the samples of two slaughterhouses and serotype 12 occurred only in the samples of one slaughterhouse.

### Genotypes

Thirty *C. jejuni* and two *C. coli* isolates were genotyped with PFGE. *SmaI* enzyme identified 14 different patterns and *KpnI* identified 15 different patterns. Together there were 18 different genotypes. The most common serotype 6, 7 was associated with *SmaI* genotype S2 (4/7) and three of the isolates were not digested with *SmaI*. All S2 isolates had highly similar patterns when digested with *KpnI* (K3) and they were named as combined genotype C3. Furthermore serotype 6, 7 isolates which were not digested with *SmaI* (UD) had identical *KpnI* patterns (K8) and they were named as combined genotype C9. All genotype C3 isolates originated from one farm from slaughterhouse 2. Genotype C9 isolates originated from three different farms but from the same slaughterhouse, 3.

Serotype 12 included two different combined genotypes C1 and C10. They were all from slaughterhouse 3 and from four different farms. One farm which had genotype C1 in July also had a positive flock in August, but the isolate was nonserotypable and its genotype was C4. The genotype C4 was also found in June, July and August. Genotype C4 was the only one which was found in the areas of all three slaughter-

houses. This genotype had serotype 27 or it was nonserotypable. Serogroup 4-complex included three different genotypes C6, C7 and C8. These types were found in two different slaughterhouses.

### DISCUSSION

Systematic monitoring on the prevalence of campylobacter serotypes and genotypes in Finnish chicken farms from the entire country was performed. There are only a few countries in Europe who monitor the prevalence of campylobacter positive flocks from the entire country. In Denmark [18] and Sweden [19] prevalences have been followed for several years. Our study period was during five summer months because it is known from several other studies that there is a clear seasonal variation in the prevalence of campylobacter positive flocks [5, 7, 8] and in the human cases [4, 6] especially within the Nordic countries, Norway, Sweden, Denmark [7, 8, 10].

The results showed that approximately 3% of the flocks were positive indicating a very low campylobacter contamination level in chickens. Previous studies have shown that Sweden and Norway also have a low campylobacter prevalence [7, 8]. According to several studies [8, 9, 20, 21] campylobacter infection is introduced sporadically into the flock from an external site in the environment. Strict hygiene and biosecurity are suggested to be one of the most successful measures against environmental contamination [8, 9, 20] and the presence of a hygiene barrier has been suggested to be the most important single biosecurity measure [10]. Moreover Gibbens et al. [22] found out in their trial, that hygiene and biosecurity measures helped to control campylobacter infection in a poultry flock.

In Finland, the poultry industry is well organized and because of a strict salmonella control programme, farmers are educated to understand the importance of biosecurity barriers and hygiene control in the prevention of environmental contamination. For example boot dips are widely used as a biosecurity barrier. The construction of chicken houses prevents environmental contamination. In addition due to cold winters, houses are well insulated thus preventing the vector animals from entering, and the inside environment may be standardized. Snow-covered earth in winter decreases the possible outside sources of contamination. Competitive exclusion, to prevent salmonella, has been in wide use for over 20 years. This also might have an impact on decreasing

colonization of campylobacters in chicken [12]. In conclusion, a combination of various preventive factors may explain the low prevalence of campylobacter positive flocks in Finland.

Only three of the farms had two subsequent campylobacter positive flocks. In Finland the poultry rearing is a batch all-in, all-out system. The empty period between flocks is approximately 2 weeks. During this time houses are cleaned, disinfected and litter is changed. Dividing the flock into cohort batches for slaughter and an empty period shorter than 14 days have been found as risk factors for campylobacter contamination [10].

Even though we only followed the prevalence of positive flocks from May to September the typical seasonal variation was seen. In May and June only a few positive flocks were identified. The prevalence increased in July and August and decreased in September. Furthermore, in humans, most domestic campylobacter infections occur in June to August [16].

The number of isolates identified was rather low, but they probably represented most of the chicken isolates circulating in the chicken food chain during this period. Heterogeneity of sero/genotypes seen in other studies [8, 23] was also found in our study. Among 30 *C. jejuni* isolates 18 combined genotypes (*SmaI* and *KpnI*) were identified. Common serotypes were subdivided into differing genotypes and certain genotypes were associated with serotypes 6, 7, 12 and 27 as also found in our earlier study [24].

Common identified serotypes were serotypes 6, 7, 12, 4-complex and serotype 27. These serotypes have also been found in our earlier studies on Finnish human *C. jejuni* isolates from 1995 and 1996 [24]. These serotypes were also seen among Finnish human and chicken isolates with known PFGE genotypes from 1997 and 1998 [25]. Our sero/genotyping studies over a 5 year period suggest that certain sero/genotypes are persistent among Finnish human and chicken isolates [6, 24, 25] and these sero/genotypes were identified in the present study as well. Further sero/genotyping studies of Finnish isolates will provide more data on the importance of these types. Simultaneous sero/genotyping of human and chicken isolates will provide data on persistent *C. jejuni* strains and their role in the contamination of broiler flocks and in human infections.

To confirm the low prevalence of *C. jejuni* within chicken farms in Finland, further monitoring studies would be needed. According to this study, sero/

genotyping indicated that certain types found among chicken isolates are persistent. Combination of sero- and genotyping can be a useful tool to follow the persistence of certain strains in the Finnish environment and circulation of certain sero/genotypes among chicken farms.

## ACKNOWLEDGEMENTS

We wish to thank Finnish poultry industry for cooperation in the collection of the isolates and Finnish Veterinary Foundation and Helsinki University Research Fund for financial support and Markku Okkonen and Urszula Hirvi for their excellent technical assistance.

## REFERENCES

1. Rohner P, Pittet D, Pepey B, Nije-Kinge T, Auckenthaler R. Etiological agents of infectious diarrhea: implications for requests for microbial culture. *J Clin Microbiol* 1997; **35**: 1427–32.
2. National Public Health Institute, Finland. National infectious disease registry. Available at: <http://www.ktl.fi/ttr/>. Accessed 8 March 2002.
3. Neimann J, Engberg J, Mölbak K, Wegener HC. Food borne risk factors associated with sporadic campylobacteriosis in Denmark. *Dansk Veterinærtidsskrift* 1998; **81**: 702–5.
4. Studahl A, Andersson Y. Risk factors for indigenous campylobacter infection: a Swedish case-control study. *Epidemiol Infect* 2000; **125**: 269–75.
5. Jacobs-Reitsma W. Campylobacter in the food supply. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*. Washington D.C.: American Society for Microbiology, 2000: 497–509.
6. Hänninen M-L, Perko-Mäkelä P, Pitkälä A, Rautelin H. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from Helsinki area. *J Clin Microbiol* 2000; **38**: 1998–2000.
7. Kapperud G, Skjerve E, Vik L, et al. Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. *Epidemiol Infect* 1993; **111**: 245–55.
8. Berndtson E. Campylobacter in broiler chickens the mode of spread in chicken flocks with special reference to food hygiene (dissertation). Uppsala, Swedish University of Agricultural Science, 1996.
9. Giessen AW van de, Tilburg JJHC, Ritmeester WS, Plas J van der. Reduction of campylobacter infections in broiler flocks by application of hygiene measures. *Epidemiol Infect* 1998; **121**: 57–66.
10. Hald B, Wedderkopp A, Madsen M. Thermophilic *Campylobacter* spp. in Danish broiler production: a cross-sectional survey and a retrospective analysis of risk factors for occurrence in broiler flocks. *Avian Pathol* 2000; **29**: 123–31.

11. Newell DG, Wagenaar JA. Poultry infections and their control at the farm level. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*. Washington D.C.: American Society for Microbiology, 2000: 497–509.
12. Shane SH. *Campylobacter* infection of commercial poultry. *Rev Sci Tech* 2000; **19**: 376–95.
13. Aho M, Hirn J. Prevalence of campylobacteria in the Finnish broiler chicken chain from the producer to the consumer. *Acta Vet Scand* 1988; **29**: 451–62.
14. Bolton FJ, Hutchinson DN, Coates D. Blood-free selective medium for isolation of *Campylobacter jejuni* from feces. *J Clin Microbiol* 1984; **19**: 169–71.
15. The Nordic Committee on Food Analysis. *Campylobacter jejuni/coli* detection in foods. NMKL Method no. 119, 2nd edn, 1990.
16. Hänninen M-L, Pajarre S, Klossner M-L, Rautelin H. Typing of human *Campylobacter jejuni* isolates in Finland by pulsed-field gel electrophoresis. *J Clin Microbiol* 1998; **36**: 1787–9.
17. Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. *Diagnostic molecular microbiology: principles and applications*. Washington D.C.: American Society for Microbiology, 1993: 563–72.
18. Danish Zoonosis Centre. Available at: <http://130.226.165.6/annualreport1999/003.html>. Accessed 8 March 2002.
19. Swedish Institute for Infectious Diseases. Annual report 1999 Available at: <http://www.smittskyddsinstitutet.se/download/pdf/rapp99.pdf>. Accessed 12 March 2002.
20. Hartnett E, Kelly L, Newell D, Wooldridge M, Gettinby G. A quantitative risk assessment for the occurrence of campylobacter in chickens at the point of slaughter. *Epidemiol Infect* 2001; **127**: 195–206.
21. Evans SJ, Sayers AR. A longitudinal study of campylobacter infection of broiler flocks in Great Britain. *Prev Vet Med* 2000; **46**: 209–23.
22. Gibbens JC, Pascoe SJS, Evans SJ, Davies RH, Sayers AR. A trial of biosecurity as a means to control campylobacter infection of broiler chickens. *Prev Vet Med* 2001; **48**: 85–99.
23. Nielsen EM, Nielson NL. Serotypes and typability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. *J Food Microbiol* 1999; **46**: 199–205.
24. Rautelin H, Hänninen M-L. Comparison of a commercial test for serotyping heat-stable antigens of *Campylobacter jejuni* with genotyping by pulsed-field gel electrophoresis. *J Med Microbiol* 1999; **48**: 617–21.