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The heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from healthy cattle

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ABSTRACT

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Aims: To identify campylobacters isolated from clinically healthy cattle at species level by a multiplex polymerase chain reaction (m-PCR). The heterogeneity among *Campylobacter jejuni* and *Campylobacter coli* isolates was also investigated by using a restriction fragment length polymorphism (RFLP) analysis of flagellin (*fla*A) gene.

Methods and Results: Samples of intestinal contents, gall bladders, liver and faeces were collected from a total number of 1154 healthy cattle. The samples were inoculated onto Preston enrichment broth and agar. Of 1154 samples, 301 (26·1%) were positive for *Campylobacter* spp. Using an m-PCR assay for species identification, 179 (59·5%) were positive with *C. jejuni* specific primers while 30 (10%) were positive with *C. coli* specific primers. None of the liver samples examined was positive for *C. jejuni* or *C. coli* by mPCR. All the isolates identified as *C. jejuni* and *C. coli* were successfully subtyped by *fla*A typing. Of the 209 isolates tested, 28 different *fla*A types were found. Twenty-three *fla*A types were identified among 179 *C. jejuni* isolates and the remaining five from *C. coli* isolates.

Conclusions: Although the overall results suggest that the degree of heterogeneity among the *fla*A genes of thermophilic *Campylobacter* strains isolated from healthy cattle is relatively high, they should be treated cautiously as the number of band types for *C. coli* was low and band type 8 in *C. jejuni* was represented by a high percentage (%58).

Significance and Impact of the Study: The findings of the present study suggest that healthy cattle can play role in the contamination of environment and human food chain by *Campylobacter* spp.

Keywords: *Campylobacter jejuni*, *Campylobacter coli*, *fla*A gene, healthy cattle, multiplex polymerase chain reaction, restriction fragment length polymorphism.

INTRODUCTION

Thermophilic *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli* are recognized as the common causes of acute bacterial diarrhoea (Skirrow 1994). In industrialized countries, *c*. 85–95% and 5–10% of *Campylobacter* infections are caused by *C. jejuni* and *C. coli* respectively (Vandamme 2000). *Campylobacter jejuni* is estimated to affect 2·5 million people per year in the USA (Mead *et al.* 1999). Although the majority of cases are sporadic, outbreaks involving consumption of contaminated raw milk and untreated water have been reported (Hanninen *et al.* 2003; Peterson 2003). The bacteria have been isolated from the faeces of farm animals including beef cattle, dairy cows and sheep (Wesley *et al.* 2000; Fitzgerald *et al.* 2001). The importance of cattle in campylobacteriosis is not just restricted to the contamination of milk at the farm and the carcass at slaughter, but they are also responsible for environmental and water contamination because of the disposal of abattoir effluents and slurries to land (Stanley

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and Jones 2003). The percentages ranging from *c*. 1 to 47% have been reported for the presence of *Campylobacter* spp. in faeces of cattle in different parts of the world (Rosef *et al.* 1983; Giacoboni *et al.* 1993).

The use of conventional bacteriological tests for the differentiation and species identification of campylobacters is often hampered by the fact that these bacteria are fastidious, asaccharolytic and possess few distinguishing biochemical characteristics (Engvall *et al.* 2002). Thus, there is a great need for simple methods for detection and reliable differentiation of thermophilic *Campylobacter* species.

However, the applicability of phenotypic methods for typing *Campylobacter* species is limited by the difficulty of obtaining standard antisera and phage reagents and lack of standardization of protocols between laboratories. Several genotypic methods developed for the typing of *C. jejuni* and *C. coli* have been reported to compare favourably with phenotypic methods (Engvall *et al.* 2002). Restriction fragment length polymorphism (RFLP) is a frequently used genotyping method that does not require a heavy demand on equipment and is also quick and cheap (Steinhauserova *et al.* 2002). However, it has a lower distinguishing value compared with, for example, comparative fingerprinting analysis by amplified fragment length polymorphism or pulsed- field gel electrophoresis (Fitzgerald *et al.* 2001). Flagellin (*fla*A) gene in *Campylobacter* spp. appears to have significant sequence heterogeneity and has been proposed as a good epidemiological marker for molecular analysis (Fischer and Nachamkin 1991). The presence of highly conserved and variable regions in *fla*A gene makes this locus suitable for PCR-RFLP analysis (Shi *et al.* 2002). Different restriction enzymes such as *Alu*I, *Dde*I, *Hin*fI, *Eco*RI and *Pst*I have been used for *fla*A typing of *C. jejuni* and *C. coli*. Among all, *Dde*I has been reported to provide the best discrimination for veterinary isolates (Ayling *et al.* 1996).

The objectives of the present study were to identify campylobacters isolated from clinically healthy Turkish cattle at species level by a multiplex PCR (m-PCR) and to investigate the heterogeneity among *C. jejuni* and *C. coli* isolates, using RFLP analysis of flagellin (*fla*A) gene.

MATERIALS AND METHODS

Sample collection

Except for faecal samples, all samples were collected from cattle slaughtered at a local abattoir in the east of Turkey between July and September 2003. Faecal samples were collected from dairy cattle at a local farm in the south of Turkey in October 2003. The geographical location of the animals, from which faecal samples were collected, was different from those sampled for internal organs. A total number of 1154 samples were collected from individual healthy cattle, which included 379 gall bladder contents, 200 intestinal contents, 325 liver and 250 faecal samples. Each individual animal was represented by only one sample, that is, no more than one sample could be collected from the same cattle. Liver and gall bladder samples were taken in sterile nylon bags and in sterile disposable syringes respectively. Faecal samples were obtained directly from the rectum of dairy cattle using sterile cotton swabs. Immediately after evisceration, the intestinal contents were collected from the lumen of the intestine using swabs which were transferred from farm or abattoir to the laboratory using tubes containing 0·9% NaCl. All the samples were transported under cool conditions (at 4°C).

Isolation of thermophilic campylobacters

Liver and gall bladder contents were inoculated directly onto Preston Campylobacter selective agar containing 7% laked horse blood (SR0048C; Oxoid, Basingstoke, UK) and Preston Campylobacter selective supplement (SR117E; Oxoid) and were incubated in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) at 42°C for 48 h.

Swab samples were aseptically transferred to 10 ml of Brucella broth (Difco, Detroit, MI, USA) containing 7% laked horse blood, Campylobacter growth supplement (SR0048; Oxoid) and Preston Campylobacter selective supplement and were then incubated at 42°C for 48 h under microaerobic atmosphere. A loop-full of broth was inoculated onto Preston Campylobacter selective agar and was incubated under the same conditions. The isolates were presumptively identified as *Campylobacter* spp. by colony appearance, microscopic morphology and motility. Then, five colonies from each positive sample were stored at −20°C within nutrient broth N.2 (CM067B; Oxoid) containing 15% glycerol.

DNA extraction

A few representative colonies from cultures were transferred into an Eppendorf tube containing 300 μl distilled water. The bacterial suspension was treated with 300 μl of TNES buffer (20 mmol l^{-1} Tris, pH 8·0, 150 mmol l^{-1} NaCl, 10 mmol l^{-1} EDTA, 0·2% SDS) and Proteinase K (200 μg ml l^{-1}) and was kept at 37°C for 2 h. Following 10 min of boiling, the same amount of phenol (saturated with Tris-HCl) was added to the suspension. The suspension was shaken vigorously by hand for 5 min and then centrifuged at 11 600 *g* for 10 min. The upper phase was carefully transferred into another Eppendorf tube and 3 mol l^{-1} sodium acetate (0·1 volumes) and 95% ethanol (2·5 volumes) were added to the suspension, which was left at −20°C overnight to precipitate the DNA. The pellet, obtained following the centrifugation at high speed for

10 min, was washed twice with 90% and 70% ethanol, respectively, each step followed by 5 min centrifugation. Finally, the pellet was dried and resuspended in 50 μl of distilled water.

Multiplex-PCR to detect thermophilic campylobacters at species level

Two pairs of primers specific to *C. jejuni* and *C. coli* described previously by Misawa *et al.* (2002) were used in m-PCR. The PCR was performed in a thermal cycler (Hybaid, Middlesex, UK) in a total reaction volume of 50 μl containing 5 μl of 10x PCR buffer (750 mmol l^{-1} Tris-HCI, pH 8·8, 200 mmol l^{-1} $(NH_4)_2SO_4$, 0·1% Tween 20), 5 μl of 25 mmol l^{-1} $MgCl_2$, 250 μmol l^{-1} of each deoxynucleotide triphosphate, 1·25 U of *Taq* DNA polymerase (MBI Fermentas, Hanover, MD, USA), 1 μmol l^{-1} of each primer and 5 μl of template DNA. PCR amplification was performed with an initial denaturation step at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min. A final extension step at 72°C for 5 min was applied. PCR amplicons were detected by electrophoresis in 2% (w/v) agarose gel and stained with ethidium bromide and then visualized under an ultraviolet transilluminator. Reference *C. jejuni* NCTC-11322 (National Collection of Type Cultures, London, UK) and *C. coli* NCTC-11366 strains were included as positive controls and distilled water was used as negative control in all the assays.

Flagellin A typing of *C. jejuni* and *C. coli* isolates

Strains identified as *C. jejuni* and *C. coli* by m-PCR were typed by PCR-RFLP for the *fla*A gene. A fragment of *c.* 1700 bp of the *fla*A gene was amplified in a PCR reaction using a pair of specific primers described by Nachamkin *et al.* (1996). All the amplicons were restricted with 2 U *Dde*I (Promega, Madison, WI, USA) in 1·5 μl 10x restriction buffer and were then incubated at 37°C for 2 h. The digested PCR products (25 μl) were separated on 2% (w/v) agarose gel. Following ethidium bromide staining, bands were photographed under UV transilluminator and the results were evaluated manually. A 100 bp DNA ladder (SM0321; MBI Fermentas) was used as molecular weight marker to evaluate the size of bands.

RESULTS

Isolation and identification of *Campylobacter* spp.

Using conventional culture method, of 1154 samples tested, 301 (26·1%) were positive for *Campylobacter* spp. The isolation rate of *Campylobacter* spp. was determined to be the highest in faecal samples with 44% (110/250) and the lowest in liver samples with 6·5% (21/325) (Table 1).

Identification of *C. jejuni* and *C. coli* by m-PCR

In m-PCR analysis of DNA samples extracted from the colonies of 301 samples, *C. jejuni* was identified from 179 (59·5%). This proportion was calculated to be 15·5% when the total number of 1154 samples was considered. The presence of *C. jejuni* was found to be the highest in gall bladder samples (85·2%), followed by faecal samples (50%). On the contrary, the isolation rate of *C. coli* was determined to be much lower with *c.* 10% of 301 samples. This proportion was calculated to be only 2·6% when the total number of 1154 samples was considered. The presence of *C. coli* was found to be the highest in faecal samples (21·8%). No *C. jejuni* and *C. coli* were isolated and identified from liver samples (Table 1).

*fla*A typing results

A total of 179 *C. jejuni* strains of which 115 were originated from gall bladders, 55 from faeces and nine from intestinal contents, were subjected to *fla*A-typing using *Dde*I. In total, 23 different types were defined by *fla*A typing (Table 2). Among the isolates originating from gall bladder samples

Table 1 Identification of *Campylobacter* spp. isolates obtained from various specimens of apparently healthy cattle by multiplex PCR (m-PCR)

Type of samples	Number of samples	Number of samples positive by culture	Number of samples (%) positive with m-PCR		
			C. jejuni	*C. coli*	*Campylobacter* spp.*
Gall bladder	379	135 (35·6)	115 (85·2)	1 (0·7)	19 (14·1)
Intestinal content	200	35 (17·5)	9 (25·7)	5 (14·3)	21 (60)
Liver	325	21 (6·5)	0	0	21 (100)
Faeces	250	110 (44)	55 (50)	24 (21·8)	31 (28·2)
Total	1154	301 (26·1)	179 (59·5)	30 (10)	92 (30·5)

Percentage values are given in parenthesis.
*The isolates that were negative in the m-PCR were considered as *Campylobacter* spp.

Table 2 Flagellin typing results of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from various specimens of cattle

Source	Species	Number of *fla*A types
Gall bladder ($n = 115$)	Cj	15
Intestinal content ($n = 9$)	Cj	2
Faeces ($n = 55$)	Cj	8
Gall bladder ($n = 1$)	Cc	1
Intestinal content ($n = 5$)	Cc	2
Faeces ($n = 24$)	Cc	3

Cj, *Campylobacter jejuni*; Cc, *Campylobacter coli.*

that yielded 15 distinct types, the most common types were type 15 (37%) and type 11 (34%) (Fig. 1). Among the isolates originating from faecal samples which yielded eight distinct types, the most common types were type 8 (58%), type 7 (15%) and type 6 (13%) (Fig. 2). The other types were represented by <6% of the isolates. All the types obtained from faecal isolates were different from those of gall bladder isolates. Two distinct types obtained from intestinal isolates were indistinguishable from types 11 and 13 of gall bladder isolates.

Of 30 *C. coli* isolates, 24 were originated from faeces, five from intestinal contents and one from gall bladder. The isolates were also subjected to *fla*A typing using *Dde*I. In total, five different *fla*A types were defined. Of these, three types were obtained from faecal samples, two from intestinal contents and one from gall bladder samples (Table 2). The latter was indistinguishable from one of the types obtained from intestinal contents. All the types obtained from faecal isolates were different from those of intestinal contents and gall bladder isolates.

DISCUSSION

The primary objective of this study was to investigate the presence of *C. jejuni* and *C. coli* from internal organs and faecal samples of clinically healthy cattle by m-PCR. The major source of humans being infected by these agents has been reported to be poultry meat contaminated with the intestinal contents of chickens (Nadeau *et al.* 2002). In recent years, researches have also been focused on farm animals in order to develop control strategies against campylobacter infection (Ono *et al.* 1995; Fitzgerald *et al.* 2001). However, most of the previous studies have investigated *C. jejuni* and *C. coli* in diarrhoeic animals such as cattle and sheep (Adesiyun *et al.* 2001; Acha *et al.* 2004), but studies related to healthy cattle are limited (Wesley *et al.* 2000). The detection of campylobacters in healthy cattle is important in order to identify possible sources of infection and to have a better understanding of the epidemiology of infection. In the present study, all the animals sampled were clinically healthy and no macroscopic lesions were observed in the internal organs to suspect of the campylobacteriosis.

Using conventional culture, 26·1% of the total samples were positive for *Campylobacter* spp. Moreover, the results of m-PCR showed that 59·5% and 10% of the isolates were identified as *C. jejuni* and *C. coli* respectively. The remaining 30·5% was considered as *Campylobacter* spp. based on routine bacteriological examination. The presence of other campylobacters (e.g. *Campylobacter lanienae*, *Campylobacter hyointestinalis)* in faeces of cattle has been reported elsewhere (Inglis *et al.* 2004). However no studies towards investigating *Campylobacter* species other than *C. jejuni* and *C. coli* have been conducted in our region and, it is therefore difficult to speculate on the species identity of these isolates. The presence of *Campylobacter* spp. in the internal organs and faecal samples of healthy cattle suggests that these animals can be major environmental reservoirs and may contaminate the environmental and human food chain. In order to determine the role of healthy cattle in environmental contamination, it is necessary to examine a variety of samples from environment and also human isolates. However, the results of this study show that a significant proportion of healthy cattle was responsible for the presence

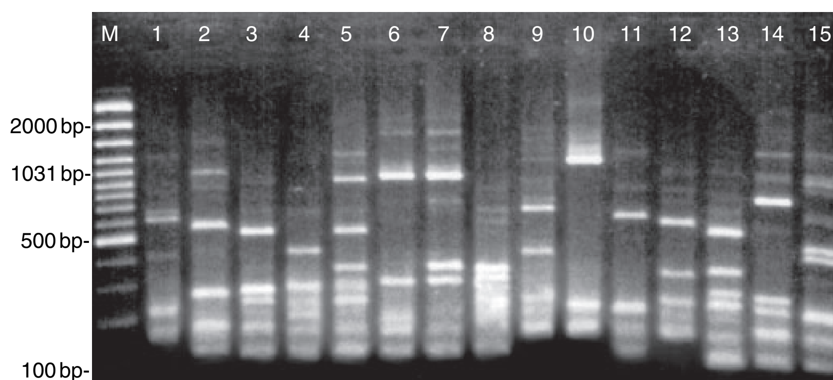

Fig. 1 Flagellin typing of *Campylobacter jejuni* strains isolated from the gall bladder samples of cattle using the restriction endonuclease *Dde*I. M: 100 bp molecular weight marker (SM0321; MBI Fermentas), lanes 1–15: different restriction types of flagellin A gene (lanes 15 and 11 represent the most frequent types)

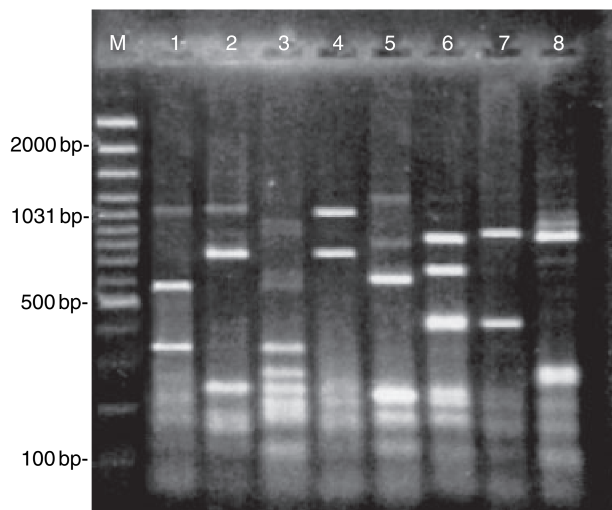

Fig. 2 Flagellin typing of *Campylobacter jejuni* strains isolated from the faecal samples of cattle using the restriction endonuclease *Dde*I. M: 100 bp molecular weight marker (SM0321; MBI Fermentas), lanes 1–8: different restriction types of flagellin A gene (lanes 8, 7 and 6 represent the most frequent types)

of *Campylobacter* species in the environment and may therefore contribute to the prevalence of campylobacteriosis in humans.

The isolation rate (35·6%) of *Campylobacter* spp. from gall bladders in this study was lower than 47% reported in a previous study carried out in the same region (Muz *et al.* 1992). Muz *et al.* (1992) examined only a limited number (112) of gall bladder samples and used different methodology for the isolation of *Campylobacter* spp. In other studies, the isolation rates of *Campylobacter* spp. were reported to vary from 16% to 90% (Ono *et al.* 1995; Stanley *et al.* 1998). A variety of factors such as age, season, number of animals examined, herd size and type and, isolation methods as well as hygienic level at the farm may be responsible for the differences in the isolation rates of the agents (Wesley *et al.* 2000). For example, in the UK, *Campylobacter* spp. was isolated from 79%, 40% and 37% of three different herds (Atabay and Corry 1998). Stanley *et al.* (1998) reported that *Campylobacter* spp. were isolated at 26·7% by direct plating and 89·4% by enrichment culture. In the current study, although liver and gall bladder samples were plated directly, an enrichment stage was applied for intestinal and faecal swabs in order to minimize the possibility of contamination and consequently enhance the chance of recovering campylobacters. As enrichment culture is generally accepted to be superior to direct plating, it can be suggested that the isolation rates of *Campylobacter* spp. from liver and gall bladder have been underestimated in this study.

Although *C. jejuni* and *C. coli* were determined to be prevalent in gall bladders and faecal samples, they could not be isolated from any of the liver samples in the present study. The isolation of *C. jejuni* and *C. coli* from gall bladders and faecal samples is not surprising, because of the fact that these agents may be found as commensals in the various organs of healthy cattle (Garcia *et al.* 1985). The isolation of *C. jejuni* and *C. coli* from the liver of other species such as chickens, sheep and pigs has been reported (Fernandez and Pison 1996; Moore and Madden 1998), but information about the isolation of these agents from clinical healthy cattle liver is rather scarce. It is noted that *C. jejuni* and *C. coli* were isolated most frequently in liver of poultry due to their appropriate body temperature which enables proliferation of these agents. However, a few studies suggested that *C. jejuni* and *C. coli* were isolated in high proportions in beef liver samples on retails which might be the result of the contamination during processing (Kramer *et al.* 2000). In the present study, it is likely that *C. jejuni* and *C. coli* were in small quantities in liver of healthy cattle and therefore could not be detected by the isolation method used here. Moore and Madden (1998) noted that a combination of direct swabbing of liver coupled with plating on both Skirrow and Blaser–Wang selective media was the most efficient combination of selective media for isolation of *Campylobacter* spp. However, in the present study, Preston Campylobacter selective agar was used for the isolation of *Campylobacter* spp.

Molecular typing of all the isolates identified as *C. jejuni* and *C. coli* were also carried out to investigate the level of genetic variations among the isolates. The presence of genetic instability because of the recombination in genetic loci including flagellin locus of campylobacters has been reported (Harrington *et al.* 1997). In this case, one of the two options may be preferred. First is the combination of several typing methods and the second is a method which is relatively stable, cheap, easy to apply and not time-consuming. *Fla*A typing is generally considered as advantageous in terms of the above-mentioned features. Using *fla*A typing, all the *C. jejuni* and *C. coli* strains isolated from intestinal contents, gall bladders and faecal samples were typeable. A total number of 28 distinct *fla*A-types were obtained. Similarly, nine to 35 *fla*A-types were identified among cattle isolates from other studies (Madden *et al.* 1998; Fitzgerald *et al.* 2001). As a matter of fact, the *fla*A-types obtained from the isolates originated from intestinal contents and gall bladders were similar to each other, whereas completely different *fla*A types were observed from those of faecal samples. This may be due to the fact that the geographical location of the animals from which faecal samples were collected was different from those sampled for internal organs. Furthermore, some of the *fla*A types were represented by remarkably high proportions among the

isolates tested. For example; *fla*A-type 8 was observed from 58% of faecal isolates. These finding should be taken into account in the investigations towards developing effective control strategies against *C. jejuni* and *C. coli* infections.

In conclusion, although the overall results suggest that the degree of heterogeneity among the *fla*A genes of thermophilic *Campylobacter* strains isolated from healthy cattle is relatively high, they should be treated cautiously as the number of band types for *C. coli* was low and band type 8 in *C. jejuni* was represented by a high percentage. The findings also suggest that healthy cattle can play significant role in the contamination environment and human food chain by *Campylobacter* spp. The comparison of molecular types of *Campylobacter* spp. originating from animals, environment and human will help to have a better understanding of the epidemiology of campylobacteriosis in humans.

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REFERENCES

Acha, S.J., Kuhn, I., Jonsson, P., Mbazima, G., Katouli, M. and Mollby, R. (2004) Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens. *Acta Vet Scand* **45**, 27–36.

Adesiyun, A.A., Kaminjolo, J.S., Ngeleka, M., Mutani, A., Borde, G., Harewood, W. and Harper, W. (2001) A longitudinal study on enteropathogenic infections of livestock in Trinidad. *Rev Soc Bras Med Trop* **34**, 29–35.

Atabay, H.I. and Corry, J.E. (1998) The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol* **84**, 733–740.

Ayling, R.D., Woodward, M.J., Evans, S. and Newell, D.G. (1996) Restriction fragment length polymorphism of polymerase chain reaction products applied to the differentiation of poultry campylobacters for epidemiological investigations. *Res Vet Sci* **60**, 168–172.

Engvall, E.O., Brändström, B., Gunnarsson, A., Mörner, T., Wahlström, H. and Fermér, C. (2002) Validation of a polymerase chain reaction/restriction enzyme analysis method for species identification of thermophilic campylobacters isolated from domestic and wild animals. *J Appl Microbiol* **92**, 47–54.

Fernandez, H. and Pison, V. (1996) Isolation of thermotolerant species of *Campylobacter* from commercial chicken livers. *Int J Food Microbiol* **29**, 75–80.

Fischer, S.H. and Nachamkin, I. (1991) Common and variable domains of the flagellin gene, *flaA*, in *Campylobacter jejuni*. *Mol Microbiol* **5**, 1151–1158.

Fitzgerald, C., Stanley, K., Andrew, S. and Jones, K. (2001) Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. *Appl Environ Microbiol* **67**, 1429–1436.

Garcia, M.M., Lior, H., Stewart, R.B., Ruckerbauer, G.M., Trudel, J.R. and Skljarevski, A. (1985) Isolation, characterization, and serotyping of *Campylobacter jejuni* and *Campylobacter coli* from slaughter cattle. *Appl Environ Microbiol* **49**, 667–672.

Giacoboni, G.I., Itoh, K., Hirayama, K., Takahashi, E. and Mitsuoka, T. (1993) Comparison of faecal *Campylobacter* in calves and cattle of different ages and areas in Japan. *J Vet Med Sci* **55**, 555–559.

Hanninen, M.L., Haajanen, H., Pummi, T., Wermundsen, K., Katila, M.L., Sarkkinen, H., Miettinen, I. and Rautelin, H. (2003) Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. *Appl Environ Microbiol* **69**, 1391–1396.

Harrington, C.S., Thomson-Carter, F.M. and Carter, P.E. (1997) Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. *J Clin Microbiol* **35**, 2386–2392.

Inglis, G.D., Kalischuk, L.D. and Busz, H.W. (2004) Chronic shedding of *Campylobacter* species in beef cattle. *J Appl Microbiol* **97**, 410–420.

Kramer, J.M., Frost, J.A., Bolton, F.J. and Wareing, D.R. (2000) *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. *J Food Prot* **63**, 1654–1659.

Madden, R.H., Moran, L. and Scates, P. (1998) Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction-restriction fragment length polymorphism and the random amplified polymorphic DNA method. *J Appl Microbiol* **84**, 703–708.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. (1999) Food-related illness and death in the United States. *Emerg Infect Dis* **5**, 607–625.

Misawa, N., Kawashima, K., Kawamoto, H. and Kondo, F. (2002) Development of a combined filtration-enrichment culture followed by a one-step duplex PCR technique for the rapid detection of *Campylobacter jejuni* and *C. coli* in human faecal samples. *J Med Microbiol* **51**, 86–89.

Moore, J.E. and Madden, R.H. (1998) Occurrence of thermophilic *Campylobacter* spp. in porcine liver in Northern Ireland. *J Food Prot* **61**, 409–413.

Muz, A., Ozcan, C., Gurcay, M. and Angin, M. (1992) Investigation on aerobic, anaerobic and microaerophilic bacteria in the gall-bladders of sheep and cattle slaughtered in meat and fish organization in Elazig. *F. U. Sag Bil Derg* **6**, 98–104.

Nachamkin, I., Ung, H. and Patton, C.M. (1996) Analysis of HL and O serotypes of *Campylobacter* strains by the flagellin gene typing system. *J Clin Microbiol* **34**, 277–281.

Nadeau, E., Messier, S. and Quessy, S. (2002) Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. *J Food Prot* **65**, 73–78.

Ono, K., Masaki, H. and Tokumaru, Y. (1995) Isolation of *Campylobacter* spp. from slaughtered cattle and swine on blood-free selective medium. *J Vet Med Sci* **57**, 1085–1087.

Peterson, M.C. (2003) *Campylobacter jejuni* enteritis associated with consumption of raw milk. *J Environ Health* **65**, 20–21.

Rosef, O., Gondroson, B., Kapperud, G. and Underdal, B. (1983) Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl Environ Microbiol* **46**, 855–859.

Shi, F., Chen, Y.Y., Wassenaar, T.M., Woods, W.H., Coloe, P.J. and Fry, B.N. (2002) Development and application of a new scheme for typing *Campylobacter jejuni* and *Campylobacter coli* by PCR-based restriction fragment length polymorphism analysis. *J Clin Microbiol* **40**, 1791–1797.

Skirrow, M.B. (1994) Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. *J Comp Pathol* **111**, 113–149.

Stanley, K. and Jones, K. (2003) Cattle and sheep farms as reservoirs of *Campylobacter*. *J Appl Microbiol* **94**, 104–113.

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. and Jones, K. (1998) The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J Appl Microbiol* **85**, 472–480.

Steinhauserova, I., Češkova, J. and Nebola, M. (2002) PCR/restriction fragment length polymorphism (RFLP) typing of human and poultry *Campylobacter jejuni* strains. *Lett Appl Microbiol* **34**, 354–358.

Vandamme, P. (2000) Taxonomy of the family Campylobacteriaceae. In *Campylobacter* ed. Nachamkin, I. and Blaser, M.J. pp. 3–27. Washington: ASM press.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. and Siddique, I. (2000) Faecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl Environ Microbiol* **66**, 1994–2000.