Epidemiological surveillance of *Campylobacter jejuni* in chicken, dairy cattle and diarrhoea patients

J. L. HUANG[1], H. Y. XU[1], G. Y. BAO[2], X. H. ZHOU[1,4], D. J. JI[1], G. ZHANG[1],
P. H. LIU[3], F. JIANG[1], Z. M. PAN[1], X. F. LIU[1] AND X. A. JIAO[1]*

[1] *Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou, Jiangsu, P.R. China*
[2] *Yangzhou First People's Hospital, Yangzhou, Jiangsu, P.R. China*
[3] *Shanghai Animal Husbandry and Veterinary Station, Shanghai, P.R. China*
[4] *Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA*

SUMMARY

Campylobacter jejuni of different subtypes were identified in chicken, dairy cattle and diarrhoea patients in China from 2005 to 2006, using multiplex PCR and RFLP. The results indicated that, of the three types of samples, *C. jejuni* was most frequently detected in poultry of the three types of samples, with an average isolation rate of up to 18·61 % and a flock contamination rate of 86·67 %. The average incidence of *C. jejuni* in overall cattle and environmental samples, milk cows, heifers and diarrhoea patients was 7·77, 5·02, 8·70 and 4·84 %, respectively. A higher prevalence was detected in outpatients than ward patients ($P < 0.01$), and in patients aged < 7 years than in older patients ($P < 0.01$). The 265 isolates of *C. jejuni* were classified into 20 distinct types by PCR–RFLP analysis of the *flaA* gene, with the genotype distribution in humans overlapping that in poultry and cattle. This suggests that certain *C. jejuni* strains circulate between humans and domestic animals such as cattle and poultry.

Key words: *Campylobacter jejuni*, chicken, dairy cattle, diarrhoea, epidemiology.

INTRODUCTION

Campylobacter is a Gram-negative, thermophilic, obligate microaerophilic bacterium, with *Campylobacter jejuni* accounting for 99 % of the reported *Campylobacter*-associated human illness in North America and the UK [1]. *C. jejuni* colonizes the intestinal mucosa of most warm-blooded hosts, including domestic animals and humans, and causes sporadic and epidemic outbreaks of gastroenteritis, particularly in immunocompromised individuals, such as patients with cancer, AIDS or diabetes, as well as very young (especially those aged < 7 years) and older people [2]. In addition, increasing evidence reveals that some serotypes of *C. jejuni* are associated with the development of Guillain–Barre syndrome (GBS) in humans [3]. *C. jejuni* is becoming the most common cause of gastroenteritis in developed countries and raises major public health concerns worldwide [4, 5]. Animals such as chicken and cattle may act as asymptomatic reservoirs, by shedding *C. jejuni* in their stools and contaminating animal food products and surface water during slaughter and carcass dressing. Various reports from Europe and the USA have demonstrated *C. jejuni* contamination in bovine faecal samples [6]. Direct or indirect cross-contamination from stools to raw milk may occur in the process of milking, which ultimately causes foodborne outbreaks of

* Author for correspondence: Professor X. A. Jiao, Jiangsu Key Laboratory of Zoonosis, Yangzhou University, 88 South Daxue Road, Yangzhou, Jiangsu 225009, P.R. China.
(Email: jiao@yzu.edu.cn)

Table 1. *Samples from diarrhoea patients*

Hospital	Ward		Age		Collection time			
	Out-patients	Hospitalized patients	<7 yr	≥7 yr	Dec.–Feb.	Mar.–May	June–Aug.	Sep.–Nov.
A	1248	611	1599	260	287	341	609	622
B	898	180	910	168	224	116	246	492
C	45	79	92	32	41	22	36	25
Total	2191	870	2601	460	552	479	891	1139

During the period July 2005–December 2006, 3061 stool specimens from patients with diarrhoea were collected from three different hospitals at Yangzhou (hospitals A, B and C). Samples sorted according to ward, patients' age and collection time.

C. jejuni after consumption of raw or inadequately pasteurized cows' milk [7]. In poultry, contamination with *C. jejuni* is commensal and is found at the highest level in the mucosal crypts of the caecum and, to a lesser extent, in the small intestine [8]. Cross-contamination of commonly consumed poultry products is one possible link between poultry and human campylobacteriosis [8]. To explore the role of poultry and dairy cows as infectious sources of *C. jejuni* in sporadic infections in China, we investigated faecal samples from poultry, dairy cows and diarrhoea patients by multiplex PCR, and classified the isolates by *fla*-PCR–RFLP.

METHODS

Sampling of human patients

During the period July 2005–December 2006, stool specimens from patients with diarrhoea were collected from three different hospitals (A, B and C) at Yangzhou, Jiangsu Province, China. Details of the samples are given in Table 1.

Sampling on cattle farms

A total of 618 rectal samples from Holstein (*Bos taurus*) milk cows, 368 rectal samples from Holstein heifers, and 545 soil and water samples were collected from 10 cattle farms in Jiangsu Province between March 2005 and June 2006. Soil samples were collected by scraping the surface soil on animal farms with wet swabs, and water samples by direct aspirating or swabs of surface water.

Sampling on poultry farms

A total of 2609 faecal samples were collected from adult chickens aged ≥6 months in 30 breeder or commercial poultry flocks in the middle, southern and eastern parts of China. These flocks included Chinese native breeds Luo-man, Ai-jiao, Pu-dong, Hai-lan, Xiao Shao-xing, Xin Yang-he, Xin-hui, Qin-ma, Non-da, Huang-ma, Guang-xi, Wu-gu, Hai-hua, Ma-hua, Ma-huang and Tu-ji. In addition, another 532 faecal samples from free-range chickens and 217 soil and water samples were collected from chicken farms using to the above procedures.

Sample preparation

Samples were taken by wiping out the cloacal or rectal contents with sterile swabs and stabbing the swabs into Cary–Blair transport medium, and transporting them to the laboratory within 24 h. All swab samples were transferred into 500 μl sterile PBS, left at room temperature for 20 min, and vortexed every 5 min to release the bacteria from the swabs. Five hundred microlitres of the suspension was added to 9·5 ml Preston Campylobacter Selective Enrichment Broth (Preston Broth supplemented with Preston Campylobacter Selective Supplement SR117, Campylobacter Growth Supplement SR84 and 5% bovine serum albumin (Oxoid, UK), and incubated under micro-aerobic conditions of 83% N_2, 10% CO_2, and 7% O_2 at 42 °C for 36 h.

DNA extraction

Bacterial cells were harvested by centrifugation and resuspended in a 1·5-ml microtube that contained 1 ml sterile double-distilled water. The suspension was adjusted to an optical density of 1·0 at a wavelength of 610 nm using a Hitachi U-1100 spectrophotometer (Eppendorf, Germany). A 400-μl aliquot of adjusted cell suspension was transferred into another micro-tube containing 0·8 μl lysozyme (50 mg/ml stock) and

Table 2. *C. jejuni* detected by mPCR assay in diarrhoea patients

	Hospital			Ward		Age		
	A	B	C	Outpatients	Hospitalized	<7 yr	⩾7 yr	Total
No. of samples	1859	1078	124	2191	870	2601	460	3061
Positive numbers	96	46	6	138	10	140	8	148
Positive rates (%)	5·16	4·27	4·84	6·30	1·15	5·38	1·74	4·84

The average positive rate of *C. jejuni* was 4·48 % in diarrhoea patients. Outpatients and patients aged <7 years had a higher prevalence rate than that of hospitalized patients and those aged ⩾7 years, respectively ($P < 0.01$). The detection rate of infection during June–August was higher than that of other months ($P < 0.05$).

incubated at 37 °C for 20 min. One microlitre of 10 mg/ml RNase, 50 μl 10 % SDS was added to the suspension and incubated at 37 °C for 30 min. One microlitre of 20 mg/ml proteinase K was added and incubated at 37 °C for 90 min. An equal volume of phenol was added to the suspension and was shaken vigorously by hand for 5 min, and then centrifuged at 12 000 rpm for 10 min. The upper phase was transferred into another microtube and genomic DNA was precipitated with 99 % ethanol and 0·3 M sodium acetate at −20 °C for 30 min. The mixture was then centrifuged at 12 000 rpm for 10 min and the upper phase was discarded. The pellet was washed with 70 % ethanol. The DNA pellet was resuspended in 100 μl sterile distilled water and stored at −20 °C as a template for the PCR.

PCR identification

Multiplex PCR (mPCR) was carried out according to the method described by Denis *et al.* [9].

Three sets of primers that targeted 16S rRNA:

forward: 5′-ATCTAATGGCTTAACCATTAAAC-3′; reverse: 5′-GGACGGTAACTAGTTTAGTATT-3′,

mapA gene:

forward: 5′-CTATTTTATTTTTGAGTGCTTGTG-3′; reverse: 5′-GCTTTATTTGCCATTTGTTTTATTA-3′,

and *ceuE* gene:

forward: 5′-AATTGAAAAATTGCTCCAACTA-TG-3′; reverse: 5′-TGATTTTATTATTTGTAGCAGCG-3′

were used for the identification of *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively. In a typical reaction, 20 μl PCR system contained 2 μl 10× PCR buffer, 3 pM of each dNTP, 20 pM 16 S1 and 16 S2 primers, 37·5 pM mapA1, mapA2, ceuE1 and ceuE2

primers, 2 μl DNA, 0·2 μl *Taq* polymerase (Takara Diagnostics, China). The reactions were performed with a thermal cycler (Bio-Rad, USA) using the following temperature-cycling parameters: initial denaturation at 95 °C for 10 min, followed by 35 cycles of amplification. Each cycle consisted of 30 s at 95 °C, 90 s at 59 °C, 1 min at 72 °C, and a final extension step of 10 min at 72 °C. Amplification generated 857-, 589- and 462-bp DNA fragments that corresponded to the genus *Campylobacter*, *C. jejuni* and *C. coli*, respectively. PCR products (8 μl) were analysed on a 1 % agarose gel that contained 0·3 μg/ml ethidium bromide in 0·5× TAE buffer and viewed under UV light. A 100-bp DNA ladder (Promega, USA) was used as a molecular size standard.

Isolation of strains

C. jejuni-positive samples by mPCR were cultured and streaked onto CCDA agar plate and incubated at 42 °C under microaerobic conditions of 83 % N_2, 10 % CO_2 and 7 % O_2 for 48 h. After incubation, light grey colonies 1 mm in diameter were subcultured and Gram-stained. The germ colonies from the subculture were then assayed for motility, production of oxidase and catalase, and other biochemical tests [9]. Finally, these isolates were assayed by hippurate hydrolysis.

PCR–RFLP assay

PCR–RFLP analysis was performed as described previously [10], with some modifications. Briefly, the oligonucleotide primers for *flaA* gene amplification have been described by Nachamkin *et al.* – forward: 5′-GGATTCGTATTAACACAAATGGTGC-3′; reverse: 5′-CTGTAGTAATCTTAAAACATTTG-3′ [10]. The PCR amplification was performed in a Biometra gradient thermal cycler (Bio-Rad), in a total volume of 100 μl, which contained 5 μl DNA, 10 μl 10× PCR

Table 3. C. jejuni *detected by mPCR assay on cattle flock farms*

Flock farm	Source of sample	No. of samples	Positive samples	Positive rates (%)	Mean of positive rates (%)
A	Milk cow	42	4	9·52	7·27
	Environment	13	0	0·00	
B	Milk cow	55	0	0·00	3·13
	Heifer	26	3	11·54	
	Environment	15	0	0·00	
C	Milk cow	51	0	0·00	1·41
	Heifer	14	1	7·14	
	Environment	6	0	0·00	
D	Milk cow	60	7	11·67	10·40
	Heifer	30	4	13·33	
	Environment	35	2	5·71	
E	Milk cow	36	3	8·33	10·00
	Heifer	16	4	25·00	
	Environment	28	1	3·57	
F	Milk cow	55	0	0·00	0·90
	Heifer	17	1	5·88	
	Environment	39	0	0·00	
G	Milk cow	115	6	5·22	13·53
	Heifer	55	17	30·91	
H	Milk cow	76	0	0·00	0·00
	Heifer	28	0	0·00	
I	Milk cow	51	2	3·92	3·80
	Heifer	28	1	3·57	
J	Milk cow	77	9	11·69	9·84
	Heifer	154	1	0·65	
	Cattle grazing area	47	3	6·38	
	Cattle bedding	40	4	10·00	
	Environment	161	44	27·33	
	Soil	16	2	12·50	
	Feed	25	0	0·00	
	Body surface	29	0	0·00	
	Milk	91	0	0·00	
Total: milk cow		618	31	5·02	
Total: heifer		368	32	8·70	
Total: environment		545	56	10·28	
Total		1531	119	7·77	

Of 1531 faecal samples collected from 10 farms, 119 (7·77%) were positive for *C. jejuni*, and of the 10 farms tested, six (60%) were positive for *C. jejuni*. The highest positive rate was up to 30·91%.

buffer, 8 μl each primer at 2·5 μM, 8 μl 2·5 mM dNTP mix, 2·5 U *Taq* polymerase (Takara Diagnostics). Thermocycling conditions included an initial hold at 94 °C for 1 min, followed by 35 cycles of 92 °C for 30 s, 55 °C for 90 s and 72 °C for 105 s. A final extension step of 72 °C for 10 min was followed by holding at 4 °C. An 8-μl aliquot of each PCR product was run on a 1% agarose gel to confirm successful amplification of the *flaA* gene. The remaining 80 μl of each PCR product was purified by using 99%

ethanol and 0·3 M sodium acetate, and resuspended in 25 μl sterile distilled water. The purified PCR product was digested with *Dde*I restriction enzyme (10 U/μl; Roche Diagnostics, Germany) in a final reaction volume of 20 μl, and kept at 37 °C overnight. The *Dde*I-digested products were detected on 2% agarose gels in 0·5× TAE buffer and photographed under UV light.

To evaluate the differentiation capability of PCR–RFLP, 10 isolates of *C. jejuni* were randomly selected

Table 4. C. jejuni *detected by mPCR assay on chicken flock farms*

Farm	Location	Breed	Flock type	Production system	Flock	No. of samples	Positive samples	Positive rates (%)
1	Province A	Luo-man	Grandparent	Farm chicken	1	30	0	0
	Province A	Ai-jiao	Grandparent	Farm chicken	2	15	11	73·33
	Province A	Pu-dong	Grandparent	Farm chicken	3	15	5	33·33
2	Province A	Luo-man	Grandparent	Farm chicken	4	50	19	38·00
	Province A	Hai-lan	Grandparent	Farm chicken	5	60	11	18·33
3	Province A	Xiao Shao-xing	Grandparent	Farm chicken	6	40	3	7·50
	Province A	Xin Yang-he	Grandparent	Farm chicken	7	9	0	0·00
4	Province A	Xin-hui	Grandparent	Farm chicken	8	130	14	10·77
5	Province B	Qin-ma	Grandparent	Farm chicken	9	247	60	24·29
6	Province B	Luo-man	Grandparent	Farm chicken	10	100	42	42·00
7	Province B	Non-da	Grandparent	Farm chicken	11	154	79	51·30
8	Province C	Qin-ma	Commercial	Farm chicken	12	166	65	39·16
9	Province C	Huang-ma	Commercial	Farm chicken	13	50	5	10·00
10	Province C	Guang-xi	Commercial	Farm chicken	14	150	56	37·33
11	Province C	Wu-gu	Commercial	Farm chicken	15	150	28	18·67
12	Province D	Hei-hua	Commercial	Farm chicken	16	229	32	13·97
13	Province D	Hei-hua	Commercial	Farm chicken	17	50	3	6·00
	Province D	Wu-gu	Commercial	Farm chicken	18	42	0	0·00
14	Province D	Ma-hua	Commercial	Farm chicken	19	121	1	0·83
	Province D	Ma-huang	Commercial	Farm chicken	20	91	1	1·10
15	Province E	Qin-ma	Parent	Farm chicken	21	100	20	20·00
	Province E	Tu-ji	Parent	Farm chicken	22	100	13	13·00
	Province E	Tu-ji	Parent	Farm chicken	23	100	10	10·00
	Province E	Huang-ji	Parent	Farm chicken	24	100	9	9·00
16	Province E	Ni-ke hon	Commercial	Farm chicken	25	90	12	13·33
	Province E	Luo-man	Commercial	Farm chicken	26	30	9	30·00
	Province E	AA+	Commercial	Farm chicken	27	20	0	0·00
17	Province E	Ni-ke hon	Commercial	Farm chicken	28	50	6	12·00
	Province E	Ni-ke hon	Commercial	Farm chicken	29	60	12	20·00
	Province E	Ni-ke hon	Commercial	Farm chicken	30	60	15	25·00
18	Province E	Tu-ji	Commercial	Free-range		523	42	8·03
Total					30	3132	583	18·61

Thirty poultry flocks were investigated. Twenty-six flocks were *C. jejuni*-positive, with a contamination rate of 86·67%. A total of 3132 cloacal swabs were collected, 583 samples were positive for *C. jejuni*, and the average positive rate was 18·61%. The percentage of *C. jejuni*-positive samples in different flocks varied from 0% to 73·33%. In addition, 6/217 (2·76%) environmental samples were positive for *C. jejuni*.

for *flaA* gene amplification. To confirm the reproducibility of this molecular method, the experiments were performed again 2 weeks later.

Statistical analysis

Significance of the clinical study was determined using Fisher's exact test. $P < 0.05$ was regarded as significant, $P < 0.01$ as extremely significant. Positive and negative predictive values were calculated by the χ^2 test.

RESULTS

PCR detection of *C. jejuni*

PCR products from reference strains of *C. jejuni* (ATCC33560), *C. coli* and *Campylobacter* spp. resulted in bands of 589 and 857 bp, 462 and 857 bp, and 857 bp, respectively. No amplification product was observed for *Escherichia coli* as a non-campylobacter control. Distinct PCR products were observed for all of the isolates.
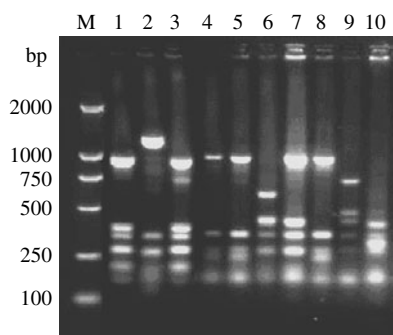
Fig. 1. Typability of *fla*-PCR–RFLP for *C. jejuni*. Lane M, DNA marker DL2000. Lanes 1–10, PCR–RFLP profiles of *C. jejuni* isolates from different sources digested with restriction enzyme *Dde*I.

Prevalence of *C. jejuni* in diarrhoea patients

Out of 3061 diarrhoea patients, 148 (4·84%) were positive for *C. jejuni*. The incidence of *C. jejuni* in hospitals A, B and C was 5·16% (96/1859), 4·27% (46/1078) and 4·84% (6/124), respectively (Table 2), but the difference between the hospitals was not significant. Of 2191 outpatients, 138 (6·30%) were positive for *C. jejuni*, while only 10/870 samples (1·15%) from ward patients were positive (Table 2). It was noteworthy that the isolation rate of *C. jejuni* from outpatients was significantly higher than that from ward patients ($P < 0.01$). In patients aged <7 years, 140/2601 (5·38%) were *C. jejuni*-positive, which was significantly higher ($P < 0.01$) than the number in patients aged ⩾7 years (1·74%, 8/460).

According to the four seasons of the year, four time periods were defined: December–February, March–May, June–August and September–November. The mean incidence of *C. jejuni* in these four periods was 3·08% (17/552), 1·67% (8/479), 6·29% (56/891), and 5·88% (67/1139), respectively. Statistical analysis indicated that the detection rate of *C. jejuni* infection during June–August was significantly higher than it was in December–February and March–May ($P < 0.05$).

Prevalence of *C. jejuni* in cattle

Of the 10 farms tested, nine (90%) farms were *C. jejuni*-positive. Of the 1531 rectal samples collected from the 10 farms, 119 samples (7·77%) were *C. jejuni*-positive, with a highest positive rate of 30·91% (Table 3). Of the total of 618 rectal samples from milk cows, 31 (5·02%) were *C. jejuni*-positive. The highest positive rate (11·69%) was detected in

farm J, and no positive results were seen in farms B, C, F and H. Out of 368 rectal samples from heifers, 32 (8·70%) were *C. jejuni*-positive. In these tested flocks, the highest positive rate (30·91%) was detected in flock G, and there were no positive animals in flock H.

Of the 545 soil and water samples from cattle farms, 56 (10·28%) were positive for *C. jejuni*. In the seven farms tested, three were *C. jejuni*-positive (42·86%). The highest contamination rate (12·96%) was detected in farm J, and no *C. jejuni* was detected in farm A, B, C or F.

Prevalence of *C. jejuni* in poultry

Out of the 30 poultry flocks, 26 (86·67%) were *C. jejuni*-positive. Of 3132 chickens, 583 (18·61%) were positive for *C. jejuni*. The prevalence of *C. jejuni* varied from 0% to 73·33% in chickens from different flocks (Table 4). The positive rate for grandparent, parent and commercial chickens was 28·71% (244/850), 13·00% (52/400) and 18·03% (245/1359), respectively. Statistical analysis indicated that the *C. jejuni*-positive rate of grandparent chickens was significantly higher than that of parent and commercial chickens ($P < 0.05$). In total, 541/2609 (20·74%) farm chickens were *C. jejuni*-positive, while only 42/523 (8·03%) of free-range chickens raised in the countryside were *C. jejuni*-positive. A difference in *C. jejuni* prevalence was also observed in different breeds, such as Ai-jiao (73·33%), Nong-da (51·30%), Luo-man (33·90%), Xin pu-dong (33·33%), and Guan-xi (37·33%) (Table 4). In addition, 6/217 (2·76%) environmental samples were *C. jejuni*-positive (Table 4).

Table 5. flaA *amplification rates of* C. jejuni *isolates from different sources*

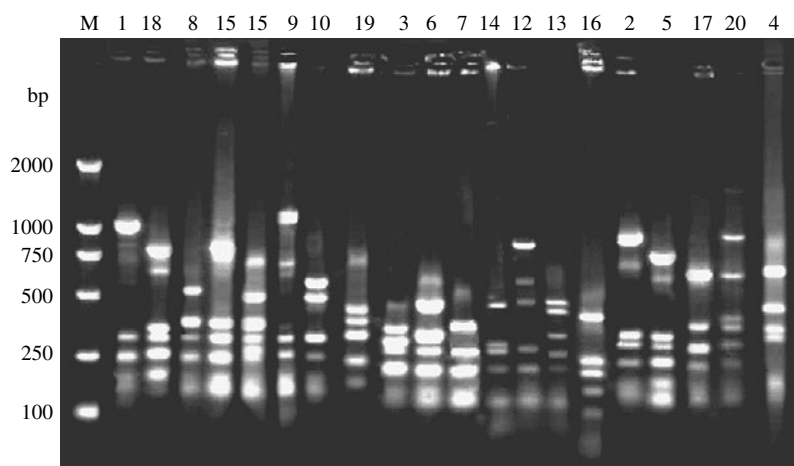|	Sources of isolates			
	Chicken	Cow	Diarrhoea patients	Total
Number amplifiable	170	25	70	265
Number unamplifiable	31	10	9	50
Amplification rate (%)	84·58	71·43	88·61	84·31

A total of 315 *C. jejuni* isolates, 79 from diarrhoea patients, 35 from cattle and 201 from poultry, were analysed by *fla*-PCR–RFLP. Two hundred and sixty-five isolates produced a *flaA*-PCR amplicon, which accounted for 84·13%. The amplified rate was the highest in human (88·61%) and lowest in cattle (71·43%) isolates.

Fig. 2. *fla*-PCR–RFLP profiles of *C. jejuni*. Lane M, DNA marker DL2000. Lanes 1–20, *fla*-PCR–RFLP profiles of selected isolates, showing 20 kinds of profile types using restriction endonucleases *Dde*I.

fla-PCR–RFLP

fla-PCR–RFLP was reproducible with a high degree of discrimination (Fig. 1). Samples 1, 3 and 7 of the 10 *C. jejuni* isolates had one band type; samples 4, 5 and 8 had another band type; and samples 2, 6, 9 and 10 had one other type each.

A total of 315 *C. jejuni* isolates, including 79 from diarrhoea patients, 35 from cattle and 201 from poultry, were analysed by *fla*-PCR–RFLP. Two hundred and sixty-five isolates produced a *flaA*-PCR amplicon of the expected 1·7-kb size, which accounted for 84·13% of the total, but the others remained untypable by this method, although all of them were confirmed as *C. jejuni* by mPCR. The highest amplified rate (70/79, 88·61%) was observed in human isolates and the lowest rate (25/35, 71·43%) in cattle isolates (Table 5). The 265 isolates, including 70 from patients, 25 from cattle and 170 from chickens, were classified into 20 distinct *fla*-PCR–RFLP profiles (Fig. 2), of which profiles 18 (12·83%), 20 (10·56%) and 5 (10·19%) accounted for >10% of the total isolates each. Furthermore, profiles 2, 4, 5, 7, 8, 11–14, 16, 18 and 20 were present in all three sample sources, accounting for 72·07% of the total isolates (Table 6).

Out of 70 human isolates, 19 different *flaA* types were identified and profile 5 was the most dominant, and accounted for 10·19% of all the human isolates. No isolates of type 1 were found. Out of 25 cattle isolates, 13 different profiles were defined, and no isolates of *flaA* types 3, 6, 9, 10, 15, 17 or 19 were detected. Out of 170 poultry isolates, 20 distinct profiles were identified, and profiles 9, 18 and 20 were the

three dominant *flaA* types, and accounted for 10·59, 16·47 and 14·12% of all the poultry isolates, respectively.

DISCUSSION

Infection with *Campylobacter* spp. is the leading cause of bacterial diarrhoea worldwide and the most common cause of travellers' diarrhoea [11, 12]. Estimated incidence of human campylobacteriosis in industrialized countries varies from 21·9/100 000 (USA) to 396/100 000 (New Zealand) [13]. It is estimated that almost all the children in developing countries have been infected once in their lifetime with *Campylobacter* spp. [14]. *Campylobacter* can be isolated from the stool for several weeks after clinical symptoms have disappeared [14]. Therefore, children infected with *C. jejuni* may become sources for transmission. Perhaps the most notable complication of *Campylobacter* infection is GBS, an important sequela of infection with *C. jejuni* strains of specific serotypes [3].

The positive rate for *C. jejuni* in this study was similar to Oyofo *et al.*'s report that 4·4% of stools were positive for *C. jejuni* in 6760 Indonesian patients with debilitating diarrhoea [15]. From the similar positive rate found in the present study, we assumed that there is a constant positive rate of *C. jejuni* in humans. However, we found that outpatients had a higher positive rate than ward patients ($P < 0.05$). This may be because most of the outpatients did not receive any antibiotics, while the ward patients were treated with appropriate antibiotics, which resulted in

Table 6. fla-*PCR–RFLP analysis of* C. jejuni *isolates from different sources*

Sub-typing	Number of isolates from different sources			Total	Percent (%)
	Chicken	Cow	Diarrhoea patients		
1	4	1	0	5	1·89
2	13	3	4	20	7·55
3	2	0	2	4	1·51
4	1	1	2	4	1·51
5	16	2	9	27	10·19
6	9	0	6	15	5·66
7	6	2	8	16	6·04
8	2	1	1	4	1·51
9	18	0	2	20	7·55
10	1	0	2	3	1·13
11	12	2	8	22	8·30
12	7	1	1	9	3·39
13	5	1	2	8	3·02
14	2	1	2	5	1·89
15	12	0	4	16	6·04
16	2	5	7	14	5·28
17	4	0	2	6	2·26
18	28	2	4	34	12·83
19	2	0	3	5	1·89
20	24	3	1	28	10·56
Total	170	25	70	265	100·00

The 265 *C. jejuni* isolates were classified into 20 distinct profiles in which profiles 2, 5, 6, 7, 9, 11, 15, 16, 18 and 20 were the most frequently observed in different sources, and accounted for 82·67%. Of 70 human isolates, 19 different *flaA* types were identified and profile numbers 5, 6, 7, 11 and 16 were the most common, accounting for 54·29%. Twenty-five cattle isolates were divided into 13 different profiles. Twenty distinct profiles were identified in 170 poultry isolates. Data presented here suggested that 12 *flaA* types were found in human, cattle and poultry isolates, which accounted for 72·07%. The results indicated that *flaA*-type distribution in human isolates overlapped with that in poultry and cattle isolates. This suggests that poultry, as well as cattle, may serve as a source of human campylobacteriosis.

a lower isolation rate. However, other factors may also have affected the detection results, such as symptom severity and disease course. In a subsequent study, we will choose appropriate patient groups in order to conduct a more in-depth investigation.

The isolation rate of *C. jejuni* from children aged <7 years was significantly higher than that of those aged ⩾7 years. This is consistent with Oyofo *et al*.'s report [15], which showed that 80% of isolates (21/26) were from children aged <5 years. Our results also displayed a significant seasonal fluctuation, with a

high isolation rate during June–August, and a lower isolation rate from December–February and from March–May. This was probably due to climatic differences. Numerous outbreaks of campylobacteriosis have been reported to be caused by torrential rain. For example, in Ontario, a large reported outbreak of campylobacteriosis was linked to the consumption of water from a municipal well [16]. Epidemiological analysis indicated that the possible source of contamination of the well was runoff from livestock following heavy rain. Bird droppings and animal waste may also be contributory factors to the contamination of surface water [17].

Campylobacter is frequently isolated from poultry and cattle, therefore effective measures to reduce or eliminate carriage of *C. jejuni* in domestic and wild animals, especially animals raised for human consumption, are essential for intervention and control of human campylobacteriosis [8]. About 7% of samples were positive for *C. jejuni* in 1531 cattle swabs in the present study, which is in agreement with the data from Dodson & LeJeune, who showed that 7% (48/686) of faecal samples from cull dairy cows was positive for *C. jejuni* [6]. However, our results on the detection rate of *C. jejuni* in cattle were lower than those from the surveillance conducted by Ono & Yamamoto who indicated that the rate of isolation of *C. jejuni* from Japanese Holstein cattle was 30·9% [18]. In Australia, there is a much wider range of carriage rates, where a median of 58% (range 12–92%) of feedlot beef cattle were *C. jejuni*-positive [19].

Our results in poultry indicated that *C. jejuni* was recovered from 18·61% (583/3132) of faecal samples. Prevalence of *C. jejuni* in poultry flocks showed considerable variation and complex epidemiology. The carriage levels varied widely in flocks distributed in different regions. However, only 2·76% of environmental samples were positive for *C. jejuni*, significantly lower than that of faecal samples. The reason for the much lower prevalence of environmental samples is probably the poor adaptive response of *C. jejuni* to environmental stress [20, 21]. The differences in carriage rates in different flocks in the present study are likely to have resulted from the periodicity of the flock raised. We also observed that different raising patterns influenced greatly the carriage rates in poultry flocks. Poultry raised on a large scale makes cross-contamination much easier. In addition, the carriage rates of different breeds showed significant variation, but the reason for this is under further investigation.

A wide range of phenotypic and genotypic typing systems have been developed and used for epidemiological typing of *Campylobacter* [22]. To detect epidemiological linkages in isolates from different sources, a typing method with high discriminatory power is extremely important. However, routine public health surveillance of *Campylobacter* spp. requires a high-throughput, low-cost method with a level of discrimination that is capable of distinguishing outbreak isolates from those occurring sporadically. Of these methods, PCR–RFLP is considered especially attractive because of its sufficient discrimination, reproducibility, relative ease of use, no requirement for specific equipment, and potential application in large-scale investigations. In addition, the results can be obtained within 24 h [10]. The key to this typing method lies in the primers designed for amplification and restriction enzymes selected for digestion. In the present study, 315 isolates confirmed as *C. jejuni* were analysed by this typing method by amplifying the *flaA* gene and digesting the amplified product with a single enzyme *Dde*I, which has been confirmed as providing the best discrimination [10]. In all the isolates, 84·13 % were successfully typed and classified into 20 distinct profiles. Profiles with the same band types as profiles 2, 4, 6, 7, 10, 11 and 16 in the present study were also reported by Petersen & On who indicated that PCR-Fla typing of the 30 isolates yielded 16 distinct genotypes [23]. Nielsen *et al.* used six typing methods on 80 *C. jejuni* isolates from poultry, cattle and sporadic human clinical cases [22]. They indicated that the dominant *flaA* types in humans were also the most common types in cattle isolates, such as serotype 2, which represented 29 % and 40 % of human and cattle isolates, respectively, but only 5 % of isolates from poultry. A study conducted by Oberhelman *et al.* has also shown that a limited number of *C. jejuni* that colonize chickens can cause disease in humans [24]. Taken together, the findings of the present and previous studies indicate that certain strains of *C. jejuni* can circulate between humans and domestic animals such as cattle and poultry. This raises public health concerns about the risk of campylobacteriosis.

ACKNOWLEDGEMENTS

DECLARATION OF INTEREST

None.

REFERENCES

1. **Tauxe RV.** Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, Tomkins LS, eds. *Campylobacter jejuni: Current Status and Future Trends.* Washington, DC: American Society for Microbiology, 1992, pp. 9–19.
2. **Park SF.** The physiology of *Campylobacter species* and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology* 2002; **74**: 177–188.
3. **Godschalk PC, et al.** PCR-restriction fragment length polymorphism analysis of *Campylobacter jejuni* genes involved in lipooligosaccharide biosynthesis identifies putative molecular markers for Guillain-Barré syndrome. *Journal of Clinical Microbiology* 2007; **7**: 2316–2320.
4. **Nachamkin I.** Chronic effects of *Campylobacter* infection. *Microbes and Infection* 2002; **4**: 399–403.
5. **Skirrow MB, Blaser MJ.** *Campylobacter*. Washington DC: ASM Press, 2000, pp. 69–88.
6. **Dodson K, LeJeune J.** *Escherichia coli* O157:H7, *Campylobacter jejuni* and *Salmonella* prevalence in cull diary cows marketed in northeastern Ohio. *Journal of Food Protection* 2005; **68**: 927–931.
7. **Jayarao BM, et al.** A survey of food-borne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *Journal of Dairy Science* 2006; **89**: 2451–2458.
8. **Hood AM, Pearson AD, Shahamat M.** The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. *Epidemiology and Infection* 1988; **100**: 17–25.
9. **Denis M, et al.** Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Letters in Applied Microbiology* 1999; **29**: 406–410.
10. **Nachamkin I, Bohachick K, Patton CM.** *Flagellin* gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* 1993; **6**: 1531–1536.
11. **Lang DR, Allos BM, Blaser MJ.** Workshop summary and recommendations regarding the development of Guillain-Barre syndrome following *Campylobacter* infection. *Journal of Infectious Diseases* 1997; **176**: 198–200.
12. **Tom H, Frieda J.** Pathogens on meat and infection in animals establishing a relationship using *Campylobacter* and *Salmonella* as examples. *Meat Science* 2006; **74**: 89–97.

13. **de Zoete MR, van Putten JP, Wagenaar JA.** Vaccination of chickens against *Campylobacter*. *Vaccine* 2007; **25**: 5548–5557.

14. **Wheeler JG, et al.** Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. *British Medical Journal* 1999; **318**: 1046–1050.

15. **Oyofo BA, et al.** Surveillance of bacterial pathogens of diarrhea disease in Indonesia. *Diagnostic Microbiology and Infectious Disease* 2002; **44**: 227–234.

16. **Clark CG, et al.** Characterization of waterborne outbreak-associated *Campylobacter jejuni*, Walkerton, Ontario. *Emerging Infectious Diseases* 2003; **9**: 1232–1241.

17. **Whyte P, McGill K, Collins JD.** An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. *Food Microbiology* 2003; **20**: 111–117.

18. **Ono K, Yamamoto K.** Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. *International Journal of Food Microbiology* 1999; **47**: 211–219.

19. **Bailey GD, et al.** A study of the foodborne pathogens: *Campylobacter*, *Listeria* and *Yersinia*, in faeces from slaughter-age cattle and sheep in Australia. *Communicable Diseases Intelligence* 2003; **27**: 249–257.

20. **Senok A, et al.** Pattern of antibiotic susceptibility in *Campylobacter jejuni* isolates of human and poultry origin. *Japanese Journal of Infectious Diseases* 2007; **60**: 1–4.

21. **Sonnevend A, et al.** High level of ciprofloxacin resistance and its molecular background among *Campylobacter jejuni* strains isolated in the United Arab Emirates. *Journal of Medical Microbiology* 2006; **15**: 1533–1538.

22. **Nielsen EM, et al.** Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry, and cattle. *Journal of Clinical Microbiology* 2000; **38**: 3800–3810.

23. **Petersen L, On SL.** Efficacy of *flagellin* gene typing for epidemiological studies of *Campylobacter jejuni* in poultry estimated by comparison with macrorestriction profile. *Letters in Applied Microbiology* 2000; **31**: 14–19.

24. **Oberhelman RA, et al.** *Campylobacter* transmission in a Peruvian shanty town: a longitudinal study using strain typing of *Campylobacter* isolates from chickens and humans in household clusters. *Journal of Infectious Diseases* 2003; **187**: 260–269.