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Seasonal variation of *Campylobacter* types from human cases, veterinary cases, raw chicken, milk and water

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J.A. HUDSON, C. NICOL, J. WRIGHT, R. WHYTE AND S.K. HASELL. 1999. During August 1996 (winter) and February 1997 (summer), a total of 180 *Campylobacter* isolates from a restricted geographical area were obtained from human and veterinary cases, raw milk and chicken, and untreated water. Isolates were typed by Penner serotyping and pulsed-field gel electrophoresis (PFGE) of restriction enzyme-produced DNA fragments. Differences were noted between the August and February serotypes, with the most, and fourth most frequently isolated serotypes in February being completely absent in August. Two other serotypes were more frequently found in the February isolates, while the reverse was true for two others. In contrast to the serotyping data, one PFGE restriction profile type was dominant in both seasons, and the pattern of distribution of isolates among the other restriction patterns was similar. Five groups of isolates in each month were indistinguishable by both typing methods. Only one group was common to both months. Another group, which was absent in August, dominated the February isolates. Marked differences in the types isolated in the two seasons were therefore evident. Some isolates from human cases were indistinguishable from others isolated from water and raw chicken, indicating possible routes of infection for humans.

INTRODUCTION

Outbreaks of campylobacteriosis are relatively rare (Pebody *et al*. 1997) and often associated with the consumption of unpasteurized milk (Evans *et al*. 1996) or untreated water (Palmer *et al*. 1983). More recently, outbreaks have been attributed to cross- or food-handler contamination in catering establishments (Kirk *et al*. 1997; Wright *et al*. 1997). Outbreaks have also infrequently been attributed to contaminated raw clams (Griffin *et al*. 1983), poultry, eggs and beef (Finch and Blake 1985). By far the most cases are, however, sporadic in nature.

While epidemiological links have been drawn between a number of risk factors and sporadic campylobacteriosis, data showing direct microbiological evidence connecting risk factors and the disease are scarce or contradictory. For example, an Australian study, which compared isolates by restriction fragment patterns (Korolik *et al*. 1995), concluded that only a small proportion of the *Camp. jejuni* strains that were present in chickens may cause human disease. In contrast, Kakoyiannis *et al*. (1988) describing the results of a New Zealand study using a similar technique, concluded that poultry appeared to be a major source of infection for *Camp. jejuni* in humans. They found that nearly half (49·7%) of the human isolates typed were indistinguishable from poultry isolates.

Campylobacteriosis is by far New Zealand's most frequently reported gastrointestinal disease. The current incidence of 285·2 cases per 100 000 per annum is around four times that of giardiasis and 7·5 times that of salmonellosis (Anon. 1998). While there have been a number of outbreaks, mostly water-borne, most investigation into the causes of campylobacteriosis has focused on sporadic cases. A case control study, known as the Multi-centre Analysis of Gastroenteritis Induced by *Campylobacter* (MAGIC) study, concluded that raw or undercooked chicken consumption was by far the most important determinant of human campylobacteriosis in New Zealand (Eberhart-Phillips *et al*.

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1995). Additional food-associated risk factors included the consumption of chicken at a restaurant, barbecued or fried chicken, unpasteurized dairy products, untreated water, particularly for those accustomed to drinking city water, and contact with cattle carcasses, live cattle, calves and puppies. A case control study in Christchurch has also been conducted (Ikram *et al.* 1994). This study also showed the consumption of chicken and the consumption of non-urban water to be risk factors, but indicated that there must be some other source of infection. These conclusions are similar to those reached in other developed countries. However, apart from the study of Kakoyiannis *et al.* (1988), published data concerning microbiological investigations into the causes of New Zealand's sporadic campylobacteriosis cases are not readily available.

To assist in drawing stronger associations between sources and cases of sporadic campylobacteriosis, and to investigate possible seasonal variation, the work described here typed isolates from environmental sources and food sources in tandem with isolates from human clinical cases. The isolation work described was carried out over two, four-week periods in winter (August 1996) and summer (February 1997), and centred on the Christchurch area.

MATERIALS AND METHODS

Media and culture methods

Oxoid media were used for the isolation of *Campylobacter*. Preston Enrichment (PE) broth was used for selective enrichment purposes, while *Campylobacter* blood-free selective agar (modified CCDA-Preston) or Skirrow agar (Skirrow 1977) was used for selective plating. Agar plates were incubated under microaerophilic conditions produced by the CampyGen™ atmosphere generation system (Oxoid). Incubation temperatures were as detailed below.

Medium term storage was achieved by keeping agar plates at 4 °C in anaerobic jars under microaerophilic conditions, by inoculating 10 ml volumes of trypticase soy broth subsequently maintained at 37 °C, or by freezing at −70 °C.

Cultures were transported when required using Difco Cultureswabs (Amies medium with charcoal) by courier. Swabs were streaked to check for purity before typing.

Identification

Identification was achieved by testing cultures for sensitivity to nalidixic acid and cephalothin (by placing 30 μg discs on Columbia blood agar plates inoculated from a broth culture by spreading using swabs), oxidase reaction using the Dry Slide™ system (Difco), hippurate hydrolysis (Lior 1984), and observing characteristic morphology and motility using phase contrast microscopy.

Isolates from human cases

Isolates were provided by a local medical laboratory over the selected sampling periods. Faeces from Christchurch residents with diarrhoea were directly plated onto *Campylobacter* blood-free selective agar and incubated at 42 °C for 48 h. Single characteristic *Campylobacter* colonies were then subcultured to ensure purity. Cultures were transported, stored and identified as described above. No details were available concerning the cases involved.

Isolates from veterinary cases

Isolates were provided by a local veterinary laboratory who supplied all of the positive *Campylobacter* isolates that were obtained during the selected sampling periods. Faeces from animals with diarrhoea occurring within 100 km of Christchurch were directly plated onto Skirrow agar. Single characteristic *Campylobacter* colonies were then either sub-cultured directly to *Campylobacter* blood-free selective agar plates or were grown in PE broth at 37 °C for 48 h. Cultures were transported, stored and identified as described above. The geographical area from which isolates were obtained was chosen to represent a predominantly rural and intensively farmed area within a distance that could reasonably be travelled from Christchurch as a day trip and so allow exposure to animal-derived contamination.

Isolates from water samples

Water sources were selected on the basis of their potential to contain *Campylobacter* on the advice of local health protection officials, i.e. no attempt was made to estimate a prevalence in local untreated water sources. Sites were sampled at least once on each of the four weeks in each month. Three sites (A-C) were rivers draining land used for predominantly agricultural purposes. Two of the sites displayed warning notices advising the public not to bathe or drink the water because of pollution concerns. Another site was a small stream draining a stock saleyard (site D), one was a pond located in a park (site E) and the last (site F) was the outflow from the city's sewage works taken at a point at the start of a three stage oxidation pond system.

On each occasion, 1 litre of water was collected and transported chilled back to the laboratory. From each sample, 500 ml was filtered through a sterile 0·45 μm pore-size filter. This filter was placed on a *Campylobacter* blood-free selective agar plate, incubated for 24 h at 42 °C, the filter removed and incubation continued for an additional 24 h. The remaining sample was filtered through a separate filter and the filter added to 100 ml PE broth, incubated for 48 h at 42 °C and then 0·1 ml spread onto a selective agar plate. This plate was then incubated for 48 h at 42 °C. Characteristic *Campylobacter*

colonies were then streaked onto an additional *Campylobacter* blood-free selective agar plate to ensure purity. Cultures were transported, stored and identified as described above.

Isolates from raw chicken

Samples (raw chicken portions) were purchased from 10 retail outlets and maintained under refrigeration for less than 24 h before analysis. The samples came from three manufacturer's packaged products, the retail outlets' own packaged products and from the outlets' delicatessen sections. Two sets of samples were obtained each week of the four-week sampling programme. A 10 g sample of raw chicken flesh was aseptically removed from the retail package, placed in a sterile Whirl Pak (Nasco, Fort Atkinson, WI, USA) bag and 90 ml PE broth added. The sample was then homogenized using a Colworth stomacher 400 (A.J. Seward, London, UK) for 1 min. The enrichment broth was incubated for 48 h at 42 °C. After incubation, a *Campylobacter* blood-free selective agar plate was inoculated with 0·1 ml of the enrichment broth. This plate was incubated microaerophilically at 42 °C and examined for growth after 48 h.

Plates showing growth were sub-cultured to selective agar to obtain single colonies which were confirmed as *Campylobacter* as indicated above.

Isolates from raw milk

Samples of raw milk were collected from farms on the day after milking. At least three samples from different farms were tested on each weekday of the sampling period. A sub-sample (0·1 ml) was spread onto a *Campylobacter* blood-free selective agar plate and incubated for 48 h. A further 10 ml sub-sample was added to 90 ml PE broth and incubated for 48 h before a 0·1 ml volume from this enrichment was spread-plated to selective agar which was in turn incubated for 48 h at 42 °C under microaerophilic conditions.

Relative timing of sampling

Sampling was carried out over four weeks in August (winter) 1996 and four weeks in February (summer) 1997. The collection of isolates from human diarrhoeal cases was delayed by one week to allow potential disease-causing strains to be consumed and produce symptoms. Repeated sampling and typing of single isolates from each sample on each occasion was considered to be more likely to result in an accurate reflection of the types contained in the samples than typing more isolates from fewer samples.

Serotyping

The Penner serotyping system was used to determine the heat stable serotypes (HS) of the strains using the passive haemagglutination technique of Penner and Hennessy (1980). Antibodies were produced in house by the methods described by these authors using their reference isolates for antibody production.

Pulsed-field gel electrophoresis (PFGE)

Analysis of DNA from the isolates was carried out by the method of Gibson *et al*. (1994). *Sma*I was used to perform the digests and gels were electrophoresed using a CHEF mapper (Bio-Rad, Ridmond, California, U.S.A.) for 22 h with a pulse time of 10–35 s.

RESULTS

Isolation of *Campylobacter*

Isolates that were hippurate-positive, with other tests supporting the identification, were regarded as being *Camp. jejuni* as most other campylobacters and related taxa are hippurate-negative (On *et al*. 1996). Other isolates were identified as *Camp. coli* on the basis that they were nalidixic acid-sensitive and hippurate-negative. It is possible that some of them were *Camp. lari* as, contrary to most schemes which indicate that *Camp. lari* is nalidixic acid-resistant, On *et al*. (1996) indicated that only 29% of isolates of this species show such resistance. It is unlikely that any of the isolates obtained from chicken were *Helicobacter pullorum* as this species requires hydrogen to grow (Atabay *et al*. 1998), and hydrogen is not produced by the CampyGen™ system.

Thirteen isolates in August and five in February were obtained from veterinary sources. They originated from a dog (one isolate), sheep (11 isolates) a pig (one isolate), cattle (four isolates) and a swan (one isolate). Overall, 65 isolates from human cases were typed with 19 isolates supplied in August and 46 in February.

Most of the winter water samples ($n = 36$) were positive for *Campylobacter*. One frequently positive site was the sewage treatment works outflow (site F) as expected. Other consistently positive sites were site B, a river draining agricultural land, and site D, a stream draining a stock saleyard. Site C was positive on five of six occasions. This site was on a large river downstream of a meat processing plant discharge and was signposted as being unfit for drinking. The pond located in a public park (site E) was positive on three of six occasions. It was selected as likely to harbour *Campylobacter* because of the presence of ducks. The last site (A) was positive on only one occasion. This was another large river draining

agricultural land. The proportions of samples found positive are given in Table 1.

Data for water samples ($n = 48$) collected in February showed a different pattern in that fewer isolates were obtained. However, the failure to isolate as many *Campylobacter* as were obtained in August cannot necessarily be attributed to an absence of the organism, as it was observed that on many occasions, selective agar plates inoculated with water samples collected in the summer were overgrown by competing organisms that may have hidden the growth of *Campylobacter*. These observations are especially true of sites E and F.

Of the raw chicken samples ($n = 113$) tested during both months, 56·6% were positive for *Campylobacter*. This figure is in agreement with the results of a larger survey of New Zealand raw poultry which determined a prevalence of 57% in 1995 (Campbell and Gilbert 1995).

Of 111 milk samples tested, only one yielded *Campylobacter*. Published data indicate that *Campylobacter* can be detected in only a small percentage of raw milk samples and so the prevalence found in this study ($<1\%$) is in broad agreement with data from others.

Penner serotyping

Table 2 shows the sources of the 65 August and 75 February *Camp. jejuni* isolates that were able to be serotyped. An additional 14 August and eight February *Camp. jejuni* isolates were not typable, a proportion similar to many other studies (Jacobs-Reitsma *et al*. 1995).

Among the August isolates, type HS4 was the most frequently isolated serotype. The 18 isolates (22·8%) of this serotype were from human cases, a sample from a cow, chicken and water. Ten isolates (12·7%) were type HS12 and originated from human, chicken and water sources. The nine (11·4%) type HS2 isolates contained representatives from human, chicken and veterinary (cow and sheep) sources, four (5·1%) type HS1 isolates from human, water and veterinary (sheep) sources, and the four (5·1%) serotype HS37 isolates were from water and veterinary (sheep) sources. Five of the isolates were of serotypes encountered only once.

In February, the most frequently occurring serotype (19·3% of isolates) was HS33. These isolates were derived from both human cases and raw chicken samples. There were 14 (16·9%) serotype HS2 isolates from human and veterinary cases (sheep), 13 (15·7%) serotype HS6 isolates from human, chicken and water sources, and 12 (14·5%) type HS21 isolates from human and chicken sources. Therefore, 66·1% of the isolates were of only four serotypes. Only six isolates were of type HS4 while the other serotypes were only detected in one or two isolates.

There were some notable changes in the proportion of isolates belonging to some serotypes between the August and February isolates. Type HS33, which was the most frequently encountered serotype in February, and type HS21, which was the fourth most frequently isolated serotype in that month, were completely absent in the August isolates. Types HS2 and HS6 occurred more frequently in February than in August, while the proportion of type HS4 complex and type HS12 isolates was reduced in the February isolates. Given that the isolation methods and locations were identical in both months, these changes in serotype either represent drift in the most prevalent serotypes or a seasonal fluctuation in serotype.

Serotypes from either month that came from human cases and were also isolated from other sources included HS1, HS2, HS4 complex, HS6, HS12, HS21, HS33 and HS38.

Pulsed-field gel electrophoresis (PFGE)

Figure 1 shows a gel representative of some of the profiles found. Table 3 shows the distribution of the PFGE restriction profiles and sources of the 73 August isolates and 64 February isolates that were typable. Six isolates from August and 19 from February were not typable as their DNA would not cut with the restriction enzymes used.

Among the August isolates, a total of 28 restriction profiles were found, 17 of which were only represented by one isolate.

Table 1 Summary of isolations from untreated waters

		Proportion positive by season	
Site letter	Location type	Winter	Summer
A	River	1/6	0/8
B	River	6/6	1/8
C	River	5/6	2/8
D	Stockyard drain	6/6	4/8
E	Pond	3/6	2/8
F	Sewage works	6/6	6/8

Table 2 Distribution of *Campylobacter jejuni* Penner serotypes found

	Isolated source									
	Human		Chicken		Water		Veterinary		Total (%)	
Serotype	August	February	August	February	August	February	August	February	August	February
1*	1	1	0	0	2	0	1 (sheep)	0	4 (5·1)	1 (1·2)
2	4	12	3	0	0	0	2 (sheep, cow)	2 (sheep)	9 (11·4)	14 (16·9)
4 Complex†	10	3	5	1	2	0	1 (cow)	1 (sheep)	18 (22·8)	6 (7·2)**
5‡	1	2	0	1	1	0	0	0	2 (2·5)	3 (3·6)
6	0	7	0	5	1	1	0	0	1 (1·3)	13 (15·7)
8§	0	0	0	0	2	0	2 (sheep, cow)	0	4 (5·1)	0 (0)
11	0	1	1	0	0	0	1 (sheep)	0	2 (2·5)	1 (1·2)
12¶	1	0	4	0	5	2	0	0	10 (12·7)	2 (2·4)
19	0	0	2	1	0	0	0	0	2 (2·5)	1 (1·2)
21	0	2	0	10	0	0	0	0	0 (0)	12 (14·5)
23, 36	0	2	3	0	0	0	0	0	3 (3·8)	2 (2·4)
27	0	0	0	2	1	0	0	0	1 (1·3)	2 (2·4)
33	0	10	0	6	0	0	0	0	0 (0)	16 (19·3)
37	0	1	0	0	3	0	1 (sheep)	0	4 (5·1)	1 (1·2)
38	1	0	1	0	0	0	0	0	2 (2·5)	0 (0)
Single isolates	1	0	0	0	0	1	2 (sheep)	0	3 (3·8)	1 (1·2)
Untypable	0	5	11	2	3	0	0	1 (swan)	14 (17·7)	8 (9·6)
Total	19	46	30	28	20	4	10	4	79 (100·1)	83 (100·0)**

* Includes serotypes 1,23 and 1,44. N.B. The combination 1,23 is not a recognized cross-reaction and indicates a mixed infection in this case.
† 4 Complex includes strains expressing any of the 4, 13, 16, 50 serotypes.
‡ Includes cross-reacting type 31.
§ Includes cross-reacting type 17.
¶ Includes cross-reacting type 42.
** One isolate from milk (serotype 4 complex) not shown in Table.
Penner serotypes 10, 15, 41 and 42,21 were only represented by one isolate. More than 100% is totalled in one column due to rounding errors.

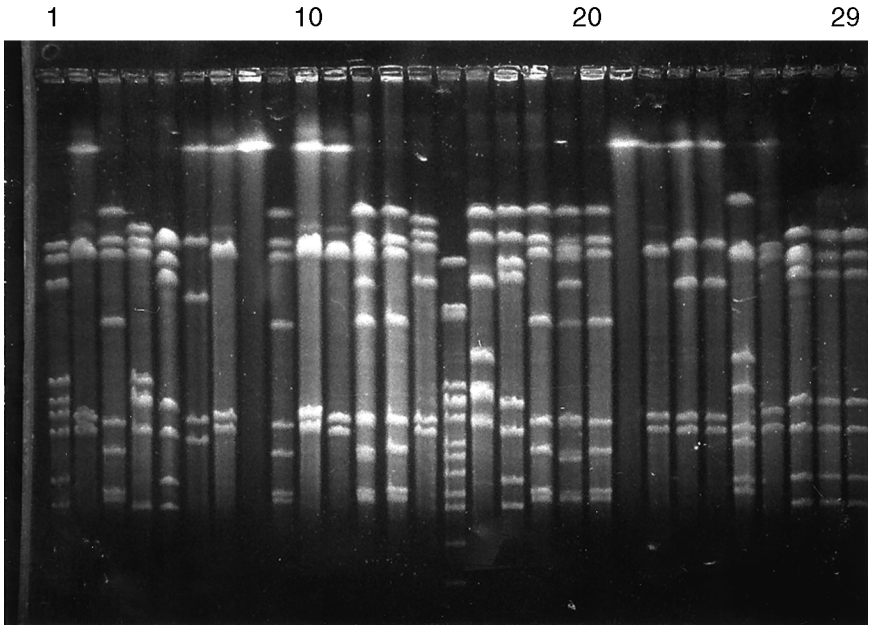

Fig. 1 Examples of 13 pulsed-field patterns obtained. Lanes 5, 27, 28 and 29: type 1; lanes 2, 7, 10, 11, 22 and 26: type 4; lanes 3, 9, 13, 18 and 20: type 19; lanes 12 and 19: type 24; lanes 23 and 24: type 25; lanes 8 and 21: untypable; lane 1: type 18; lane 4: type 20; lane 6: type 21; lane 14: type 29; lane 15: type 30; lane 16: type 22; lane 17: type 23; lane 25: type 26

Restriction profiles 1 and 20 were detected in isolates only from humans. Restriction profile 24 was found in two isolates from chicken. Isolates of the most frequently detected profile, type 25, were mostly from chicken (11 isolates) with two other isolates coming from water and one from a human case. No single restriction profile was found in isolates from all sources, but profile type 3 isolates were detected from human, chicken and veterinary (sheep) samples while profile type 4 isolates were from human, chicken and water sources.

In a similar manner to the August data, most (33·7%) of the February isolates were of profile 25, with isolates coming from human, chicken and water samples. Again, profiles 1 and 20 A, B and C were mostly from humans (one profile type 20 isolate came from a sheep sample). Most profile type 18 A and 18 B isolates (10 from 11) also came from human cases.

Unlike the serotypes, the distribution of PFGE restriction profiles seemed to be more consistent within isolates sampled at different times of the year, with profile 25 being the most frequently detected in both sets of samples. Predominant restriction profiles were therefore shared by many serotypes. Human and other sources both yielded profile type 4 isolates in both months.

Combinations of serotypes and PFGE restriction profile types represented by more than two isolates

Table 4 shows that among the August isolates, only five groups of more than two *Camp. jejuni* isolates were indistinguishable by both typing methods. These groups are denoted by the designation serotype : restriction profile type. Types 4 : 1 and 2 : 3 are interesting as all eight isolates of these types were of human origin. Isolates of types 12 : 4 and 4 : 19 were from human, chicken and water sources. In the case of the 12 : 4 isolates, three of the four water isolates came from a stream draining a stockyard, and so it is likely that these isolates were shed by animals housed at the stockyard. Type 2 : 18 was represented by three isolates, two from chicken and one from a sheep sample.

Only type 2 : 18 was common to both the August and February isolates (in addition to type 2 : 18 A which is very similar). While type 2 : 18/2 : 18 A was found in both months, the sources of isolates within this group changed from two chicken and one veterinary (sheep) isolate in August to almost all (nine from 10) human isolates in February. The dominant combination among the August isolates was 33 : 25, and the most common PFGE profile identified in the indistinguishable August groups was 25.

Only two isolates, one from water and the other from a swan, were untypable by both methods. These had both been confirmed as *Camp. jejuni* according to the characteristics listed above.

Typing of *Camp. coli*

Only two of the nine August *Camp. coli* isolates (one human, three veterinary comprising one each from a dog, pig and a sheep, one chicken and four from water) showed a shared type. The isolates from a dog and a pig were both restriction profile 9 but were Penner serotype untypable. The literature indicates that pigs carry *Camp. coli* rather than *Camp. jejuni*.

In February, one human, one chicken, one veterinary (cow)

Table 3 Distribution of *Campylobacter jejuni* PFGE restriction profiles found

	Isolate source									
	Human		Chicken		Water		Veterinary		Total (%)	
Profile	August	February	August	February	August	February	August	February	August	February
1 and 1A	5	3	0	0	0	0	0	0	5 (6·3)	3 (3·6)
3	4	0	2	0	0	0	1 (sheep)	0	7 (8·9)	0 (0)
4	2	1	4	2	5	1	0	0	11 (13·9)	4 (4·8)
11	0	0	0	0	2	0	0	0	2 (2·5)	0 (0)
16	0	0	1	0	0	0	1 (sheep)	0	2 (2·5)	0 (0)
18, 18A and 18B	0	10	2	0	0	0	1 (cow)	1 (sheep)	3 (3·8)	11 (13·3)
19	1	0	4	0	1	0	0	0	6 (7·6)	0 (0)
20, 20A, 20B and 20C	2	5	0	0	0	0	0	1 (sheep)	2 (2·5)	6 (7·2)
22 and 22A	0	2	0	1	0	0	1 (sheep)	0	1 (1·3)	3 (3·6)
23, 23A and 23B	0	0	0	1	0	0	1 (sheep)	0	1 (1·3)	2 (2·4)†
24	0	0	2	0	0	0	0	0	2 (2·5)	0 (0)
25	1	12	11	15	2	1	0	0	14 (17·7)	28 (33·7)
26	1	0	1	0	0	0	0	0	2 (2·5)	0 (0)
27 and 27A	0	0	1	0	1	0	0	0	2 (2·5)	0 (0)
Single isolate types	2	4	1	0	5	2	5 (2 cow, 3 sheep)	1 (sheep)	11 (13·9)	7 (8·4)
Untypable	1	9	1	9	4	0	0	1 (swan)	6 (7·6)	19 (22·9)
Total	19	46	30	28	20	4	10	4	79 (100)	83 (100)†

Letters indicate that the profile was very similar to (differing by one band), but not identical with the banding profile obtained by other strains with the same number (e.g. 22, 22A, 22B and 22C).

† One isolate from milk (typing in the 23/23A/23B group) not shown in Table.

Patterns 5, 6, 8, 9, 10, 12, 13, 15, 17, 28, 29, 34, 35 all contained one isolate from the August samples. Patterns 16, 21, 29, 31, 32, 33 and 36 all contained one isolate from the February samples. Totals include those single isolates not listed.

Table 4 Combinations of *Campylobacter jejuni* isolates indistinguishable by both typing methods and represented by more than two strains

Type	Number of isolates
	August
2 : 3	Human (3)
2 : 18	Chicken (2), Veterinary (1)
4 : 1	Human (5)
4 : 19	Human (1), Chicken (4), Water (1)
12 : 4	Human (1), Chicken (4), Water (4)
	February
2 : 18	Human (4), Veterinary (1)
2 : 18A	Human (5)
6 : 25	Chicken (2), Water (1)
21 : 25	Chicken (3)
33 : 25	Human (9), Chicken (5)

The numbers shown for the type represent the serotype separated from the pulsed-field pattern by a colon. Numbers in brackets represent the number of strains of each type coming from a particular source.

and six water isolates were identified as *Camp. coli*. One isolate from chicken and one cow isolate were serotype HS6. The veterinary isolate of this pair was restriction profile 1 but the chicken isolate was untypable. All of the other isolates were untypable by one or both methods.

Of note was the large proportion of *Camp. coli* isolates from water in February, where five from nine *Campylobacter* isolates were identified as belonging to this species. This predominance was not noted in the August isolates. The distribution of isolates among the types found was quite scattered and so, for this species, the data indicate there to be no strong connection between strains found in humans and those found in other environmental sources. However, the number of isolates obtained was very small.

DISCUSSION

The seasonal nature of the incidence of campylobacteriosis is a well documented phenomenon (e.g. Brieseman 1990) and not one that is easily explained by the survival of the organism in the environment. *Campylobacter* has been shown to be present in shellfish (Teunis *et al.* 1997) and surface waters (Jones *et al.* 1990a) in a manner which is negatively correlated with peaks in incidence of the disease. However, possible positive correlations seem to exist with prevalence in various sites in animal intestines (Wallace *et al.* 1997; Stanley *et al.* 1998a) and faeces of dairy cattle (Stanley *et al.* 1998b). Similar observations have been made in New Zealand dairy cow rectal swabs (Meanger and Marshall 1989). A positive correlation with numbers in sewage sludge (Jones *et al.* 1990b) presumably reflects effect rather than cause.

The typing data obtained clearly indicate a difference in the strains of *Camp. jejuni* that were isolated in the summer and winter. However, as the study was carried out over only one year, it is not certain whether there is a distinct seasonal change in extant strains, or whether there is merely drift over time. It is interesting to note that type 33 : 25 which was absent in winter was the dominant type found in summer. In addition, the proportion of PFGE restriction profile 25 isolates approximately doubled (from 17·7% to 33·7%) from August to February, which may indicate that the frequency of the 33 : 25 strain occurring in summer was superimposed over a background proportion of restriction profile 25 isolates. It is therefore possible that the emergence of the 33 : 25 strain is linked to the spring/summer peak in incidence of campylobacteriosis as it was found in both human cases and chicken samples, but this would require confirmation.

Guillain Barré Syndrome, which is a potentially fatal neurological complication of *Campylobacter* infection, has been associated with, but not exclusively to, Penner serotype HS19 isolates (Allos and Blaser 1994). Among the isolates tested, three HS19 isolates were found, and all of these came from raw chicken samples.

A significant proportion of the isolates found in both months were serotypes HS2 or HS4 complex. Other studies have also found serotypes HS2 and HS4 complex to be common among *Camp. jejuni* strains in, for example, faeces, meat and offal (Fricker and Park 1989), and poultry and livestock (Jones *et al.* 1984). It might have been expected from published data that a significant proportion of HS1 and HS3 isolates would have been detected, but this was not so as only five HS1 and no HS3 isolates were found among all of the isolates.

Serotypes which were detected among the August isolates included HS12 and HS37. However, to the knowledge of the authors, serotypes HS12 and HS37 have not been reported as frequent isolates in other studies, or in other New Zealand clinical strains (data not shown). These two serotypes may be associated with the Christchurch region of the country.

Serotype HS33, the most common serotype among the February isolates, is infrequently reported in the literature although it has been listed among strains isolated from water (Martikainen *et al.* 1990). Serotypes HS6 and HS21, also frequently found among the February isolates, have been isolated from poultry (Jones *et al.* 1984; Hood *et al.* 1988), dogs (Jones *et al.* 1984) and water (Bolton *et al.* 1987; Martikainen *et al.* 1990).

Some isolates from water were indistinguishable from human and chicken isolates in the August data (types 4 : 19 and 12 : 4). While the data do not show unequivocally that the vehicle for the human cases was either chicken or water, they do show that isolates from either of these two sources

are indistinguishable from isolates capable of producing disease in humans. If *Campylobacter* strains from raw chicken are a significant cause of sporadic cases, then isolates of the same strain from water should be equally capable of causing disease. The potential for *Campylobacter* from water to cause human disease is known in outbreaks but is less well recognized in sporadic cases. To better assess the risk that contaminated water poses it is desirable to obtain data on the distribution and numbers of *Campylobacter* present in recreational water sources.

Epidemiological studies indicate that the greatest risk factor for campylobacteriosis is the consumption of chicken. Three types (4 : 19, 12 : 4 and 33 : 25) were only found in human case and chicken product isolates, and these types constitute the most numerous indistinguishable groups of isolates. These data support the conclusions from epidemiological studies that raw chicken could act as a vehicle for human sporadic cases. Although this is not unequivocally shown by the data obtained, the balance of probability would suggest that humans would be more likely to acquire infection from raw chicken than the other way round. However, an alternative explanation of these data is that both humans and chickens acquire *Campylobacter* from a common unidentified source, and this possibility is not excluded by the data.

This study has found that some types which are indistinguishable by both methods (types 2 : 3, 4 : 1, 2 : 18/2 : 18 A) were exclusively or predominantly isolated from human cases. The question arises as to what the source(s) of infection in these cases is. There is a possibility of person-to-person spread and this has been noted in secondary cases associated with outbreaks (Stuart *et al*. 1997). There may be an unidentified environmental reservoir which could be a single food, or perhaps a range of foods that infrequently contain *Campylobacter*. An interesting observation is that type 4 : 1 was considered to be the outbreak strain in an outbreak in Auckland (New Zealand) in the Spring of 1996. The cause of this outbreak was never identified (Anon. 1996).

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