

## Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis

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### SUMMARY

Consumption of milk contaminated with *Campylobacter jejuni* has been described as a cause of human enteritis. Although faecal contamination of milk with the organism has frequently been described, direct milk excretion of *Campylobacter jejuni* into milk has rarely been linked with cases of human infection. We describe the investigations undertaken following the isolation of *Campylobacter jejuni* from samples of unpasteurized milk prior to retail. Results of epidemiological investigations including typing of *Campylobacter jejuni* isolates using pyrolysis mass spectrometry, Penner and Lior serotyping, biotyping, phage typing and restriction fragment length polymorphism analysis provided convincing evidence implicating direct milk excretion of *Campylobacter jejuni* by one asymptomatic dairy cow as the source of the milk contamination and the cause of local cases of human enteritis.

### INTRODUCTION

Many outbreaks of *Campylobacter jejuni* enteritis have been associated with the consumption of unpasteurized or inadequately pasteurized cow's milk [1–4]. Surveys have shown 0–6% of raw cow's milk to be contaminated with *C. jejuni* [5, 6]. It is believed that the most frequent source of campylobacter in raw milk is faecal contamination from cattle infected or colonized with the organism, as it

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is known to be a common commensal of their gastrointestinal tracts [5, 7]. Since the demonstration of experimentally induced *C. jejuni* mastitis in cattle [8] however, and the reporting of naturally occurring cases of campylobacter mastitis [9, 10] it has been recognized that occasionally cattle may excrete campylobacter directly into milk as a result of mammary infection. To date, this source of milk contamination has rarely been implicated in human cases of campylobacter enteritis [2, 3, 10].

We describe the investigations undertaken following the isolation of *C. jejuni* from samples of unpasteurized milk prior to sale by a raw milk producer-retailer. Pyrolysis mass spectrometry (PyMS) was used as a rapid screening method to identify the likely source of milk contamination and to determine whether there was any evidence of a milk-born human outbreak. Isolates of *C. jejuni* from our investigations were then typed by Penner and Lior serotyping, biotyping using the modified Preston scheme, phage typing and restriction fragment length polymorphism analysis (RFLP).

## METHODS

### *The background*

It has been the practice of one Environmental Health Department in our region to sample all supplies of unpasteurized milk on a regular basis. On 10 March 1992, one such routine sample of raw cow's milk was found to be positive for *C. jejuni*. A subsequent sample collected from the same supplier 7 days later was negative, but on 14 April 1992, *C. jejuni* was isolated again. Although there had been no recently perceived increase in human cases of campylobacter enteritis, in response to this risk to public health, a Pasteurisation Order was issued preventing the producer-retailer from selling unpasteurized milk pending the outcome of further investigations.

### *The dairy*

The dairy herd consisted of 44 Friesian-Holsteins and 2 Jersey cattle, 31 of which were in milk at the time of the investigation. No recent illness had been noted in any member of the herd. Each day, the business delivered approximately 200 pints (110 l) of unpasteurized milk to the local village and approximately 77 gallons (350 l) of milk was sent to the Milk Marketing Board for pasteurization prior to retail.

### *Investigations*

Case finding was encouraged by contacting the local general practitioners, who agreed to submit faeces samples to their local microbiology laboratory from all patients presenting with diarrhoea. Briefly, local Environmental Health Officers (EHOs) visited 44 people who complained of diarrhoea of onset between 9 and 29 April. Stool samples were obtained from 16 patients.

A site visit was carried out with the help of the Newcastle Veterinary Investigation Centre and the dairy was found to be in good general order. The milking parlour, which utilized a 24-point pipeline system, and the dairy were housed in the same building, but separated by a communicating door. The milking

equipment was relatively new, and a cleaning cycle which included a hypochlorite disinfection stage was being implemented. Certain problems were, however, identified. At the time of the investigation, the cattle were permanently housed in the milking parlour, and there was marked environmental faecal contamination. General advice was given about cleanliness of personal clothing, the use of hygienic disposable gloves when assembling milking equipment and inserting the milk sock, and the cleaning and disinfection of teats prior to milking was instituted. Further samples of milk were collected in the month following the implementation of these improvements. Despite a general reduction in the total viable counts, these milk samples remained positive for *C. jejuni* and the Pasteurisation Order could not be removed. Additional investigations were therefore undertaken.

A 900 ml sample of water was collected from a pre-wash rinse of the milking pipeline after milking, and examined for the presence of *C. jejuni*. The udders and teats of ten cattle were sampled with sterile swabs before cleaning and disinfection. These were examined for the presence of campylobacter to determine whether faecal contamination of udders might be the source of the organism in the milk. In order to test for gastrointestinal carriage of campylobacter in the herd, individual faeces samples were collected onto sterile swabs from the rectal perianal areas of ten cattle. On 1 June 1992, individual samples of foremilk were carefully collected from each of the 31 cows in milk prior to the evening milking. The teats of each cow were first cleansed with 70% alcohol which was allowed to dry before samples of milk from each quarter were pooled. Approximately 30 ml milk was collected from each cow into a sterile universal container. Additionally, a sample of bulk milk was collected aseptically. These samples were processed as rapidly as possible on arrival at the laboratory the same evening.

Results revealed that the foremilk sample of one cow (cow 358) and the bulk milk sample were positive for *C. jejuni*, while the other 30 foremilk samples were negative. The suspect cow's milk was therefore excluded from subsequent bulk milk collections and follow-up samples of both bulk milk and cow 358's foremilk were obtained on 8 June 1992.

#### *Bacteriological methods*

##### *Milk samples*

Enrichment culture for *C. jejuni* was performed on all milk samples. For the first milk sample examined, 25 ml of milk was added to an equal volume of double strength Preston Campylobacter Selective Enrichment Broth (Unipath Ltd), incubated for 24 h at 42 °C and then subcultured onto Campylobacter Blood-free Selective Medium (modified CCDA-Preston, Unipath Ltd) before incubation in 5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub> for 48 h at 42 °C. For all subsequent samples, 25 ml milk was added to 75 ml single strength Preston Campylobacter Selective Enrichment Broth before processing as above. When quantitative culture was carried out, 0.1 ml of a well-mixed sample of milk was inoculated directly onto, and spread over a modified CCDA-Preston agar plate which was then incubated for 48 h at 42 °C microaerobically and the resulting colony count recorded. Enrichment culture of the wash-water sample from the milk-pipeline was performed as above.

*Udder swabs*

Each swab was inoculated into 10 ml single strength Preston *Campylobacter* Selective Enrichment Broth and incubated for 24 h at 42 °C. Subcultures were then made onto modified CCDA-Preston media and incubated as above.

*Cattle faeces*

Rectal/perianal swabs were inoculated directly onto modified CCDA-Preston media, incubated microaerobically for 48 h at 42 °C and examined for the presence of *Campylobacter* spp.

Suspect colonies were provisionally identified as *C. jejuni* by the characteristic appearance on Gram-staining using 0.3% Carbol Fuchsin as counterstain, and by the results of an oxidase test, hippurate hydrolysis and susceptibility to a 30 µg nalidixic acid disk [11]. Identification was confirmed by biotyping using the modified Preston scheme [12].

*Typing of isolates*

A total of 28 isolates of *C. jejuni* from bulk milk samples (18 isolates), the foremilk of cow 358 (2 isolates, collected 1/6/92 and 8/6/92), rinse water from the milk pipeline in the dairy (1 isolate), cattle faeces (4 isolates) and from possibly associated human cases (3 isolates) remained viable for typing. For comparative purposes, 16 strains of *C. jejuni* from unassociated, sporadic clinical cases were typed simultaneously.

Isolates were stored using Microbank Beads (Pro-Lab Diagnostics). However, because we did not have the facilities for storage of cryovials at -70 °C as recommended by the manufacturer, we had to compromise with storage at -20 °C. Unfortunately, we experienced difficulty in maintaining viability at this temperature, and therefore not all isolates were available for typing by all six methods.

PyMs analysis was carried out to provide rapid screening, in order to determine whether the isolates appeared closely related. Preliminary PyMS investigations suggested that many of the isolates of *C. jejuni* were similar but not necessarily indistinguishable by this method and so, in order to make a more accurate assessment of relatedness, multiple subcultures of some isolates were pyrolysed. All were grown on blood agar plates incubated at 42 °C microaerobically overnight. Triplicate samples were pyrolysed in a single machine-run at 530 °C on a Horizon Instruments 200X pyrolysis mass spectrometer (Horizon Instruments Ltd, Heathfield, Sussex) as previously described [13]. Integrated ion counts at unit mass intervals of 51–200 were recorded and, after normalization to correct for variations in sample size, replicate spectra from each subculture were labelled as distinct groups and analysed for between-group to within-group variation. The 150 mass ions, ranked for discrimination, were used in principal component (PC) and canonical variate (CV) analysis with differences being expressed as a series of Mahalanobis distances. These data were used to construct ordination diagrams of PCCV1 v PCCV2, which were inspected for evidence of clustering. The apparatus, techniques and principles involved have been described previously [14]. In addition, ten subcultures of one isolate from the bulk milk (isolate K) were

compared with ten of a strain isolated from the foremilk of cow 358 (isolate L) in a one-to-one analysis, as documented elsewhere [15]. The difference between the means of the two groups was equivalent to a Chi-squared value with one degree of freedom.

After PyMs analysis had suggested that many of the isolates were closely related, further typing schemes were applied in order to characterize the isolates as precisely as possible. The other typing schemes applied have been described elsewhere and comprised Penner [16] and Lior [17] serotyping, RFLP fingerprinting [18], biotyping using the modified Preston scheme [12] and bacteriophage typing [19].

## RESULTS

### *Culture results*

Nineteen samples of bulk milk were examined between 10 March and 29 May 1992. Despite an overall reduction in total viable count over time consistent with the general improvements in hygiene, 18 of the 19 samples continued to yield a growth of *C. jejuni*. (Results not shown.) Examination of rinse water from the milk pipeline after milking, effectively a sample of bulk milk diluted with water, also demonstrated the presence of *C. jejuni*. Only 1 of the 31 individual foremilk samples collected from the cows in milk on 1 June 1992, that collected from cow 358, was positive for the organism. *C. jejuni* was also isolated from a bulk milk sample collected at the same time. Repeat testing of cow 358's foremilk, on 8 June 1992, confirmed direct milk excretion of *C. jejuni* and after exclusion of this cow's milk from the milk collection, subsequent bulk milk samples became negative for the organism. Gastrointestinal carriage of *C. jejuni* was demonstrated in 6 of the 10 cattle tested, while swabs collected from the udders and teats of 10 cattle were all negative for the organism. *C. jejuni* was not isolated from the faeces or udder swab collected from cow 358.

*C. jejuni* was isolated from 7 of the 16 stool specimens collected from patients who presented to their General Practitioners with diarrhoea during the period of the investigation. All seven admitted recent consumption of raw milk supplied by the implicated dairy.

### *Typing results*

In the initial PyMS analysis two unrelated clinical strains and one from cattle faeces were differentiated from the remainder. This spectral data is shown in Fig. 1. Subsequently, five further isolates were distinguished by PyMS. These comprised three sporadic human isolates, one from cattle faeces and one from a bulk milk sample. The remaining 24 isolates which underwent PyMS analysis were indistinguishable by this method. These isolates included one clinical strain thought to be associated with the outbreak (isolate T), two isolates from cattle faeces (isolates P and Q), several isolates from bulk milk samples (isolates B to K), cow 358's foremilk (isolate L) and one strain isolated from an apparently unrelated human case (isolate V).

The one-to-one PyMS analysis performed on replicate spectra from one bulk milk sample isolate (isolate K) and one isolate from cow 358's foremilk (isolate L) gave a PyMS difference of 3.036. As this was within the 95% confidence limit, these two isolates were indistinguishable by PyMS.

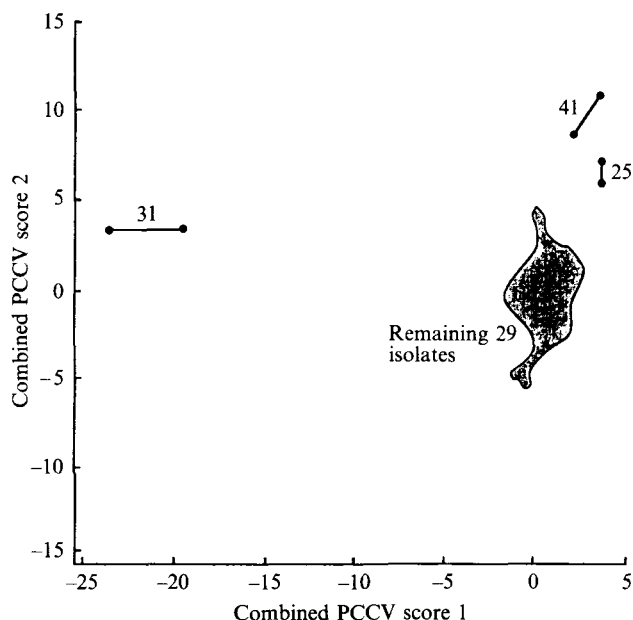


Fig. 1. Ordination diagram of spectral data of *C. jejuni* isolates. The axes represent the first two canonical discrete functions. The means of triplicate analysis of subcultures of the same isolate have been joined together. S, isolates from unassociated human cases; CF, isolate from faeces of cow 513.

The source, biotype, phage group, Lior and Penner serotyping, RFLP and PyMS analysis results for relevant isolates of *C. jejuni* are given in Table 1. PyMS analysis has been reported as 'distinct' where PyMS has distinguished isolates as being different, or 'cluster' where the strains were indistinguishable. Distinct RFLP patterns were assigned an RFLP number by comparison with the type strain patterns of the Manchester database.

Biotyping using the modified Preston scheme, phage typing, Penner and Lior serotyping and RFLP analysis supported the results obtained by PyMS analysis of the viable *C. jejuni* isolates except on four occasions. The combination of typing methods successfully distinguished 14 of the 16 strains isolated from unassociated human cases of enteritis. All six typing methods identified isolates from samples of bulk milk, cow 358's foremilk, milk pipeline rinse water and faeces from one cow as indistinguishable. This strain was characterized as biotype 6010, serotype Lior 1 or Lior non-typable, Penner 2, and 'phage group 52'. RFLP analysis showed a distinct pattern characteristic of RFLP type 2.

Only isolate T, of three possibly associated human cases of enteritis, was indistinguishable from the above strains by PyMS. The remaining two isolates (R and S) unfortunately failed to grow on subculture for PyMS analysis during the initial machine-run, but were shown to be indistinguishable by the other five typing schemes. Two human cases of *C. jejuni* enteritis were therefore shown to be caused by an isolate indistinguishable from that found in the bulk milk sample, the individual foremilk of one cow and the faeces of at least one other member of the dairy herd.



Table 1. Typing results for 23 *C. jejuni* isolates

Isolate	Source*	Biotype	Phage group	Serotypes†	RFLP	PyMS
A	Bulk milk	6010	52	P2, L1	2	Distinct
B	Bulk milk	6010	52	P2, L1	2	Cluster
C	Bulk milk	6010	52	P2, L NT	2	Cluster
D	Bulk milk	6010	52	P2, L NT	2	Cluster
E	Bulk milk	6010	52	P2, L NT	2	Cluster
F	Bulk milk	6010	52	P2, L NT	2	Cluster
G	Bulk milk	6010	52	P2, L1	2	Cluster
H	Bulk milk	6010	52	NV	2	Cluster
I	Bulk milk	6010	52	P2, L1	2	Cluster
J	Bulk milk	6010	52	P2, L NT	2	Cluster
K	Bulk milk	6010	52	P2, L1	2	Cluster
L	Foremilk – cow 358 (1/6)	6010	52	P2, L1	2	Cluster
M	Foremilk – cow 358 (8/6)	6010	52	P2, L1	2	NV
N	Pipeline	6010	52	P2, L1	2	Cluster
O	Faeces – cow 444	6010	52	P2, L1	2	Distinct
P	Faeces – cow 471	6010	52	P2, L NT	2	Cluster
Q	Faeces – cow 369	6000	44	NV	NV	Cluster
R	Associated	6010	52	P2, L NT	2	NV
S	Associated	6010	52	P2, L NT	2	NV
T	Associated	NV	NV	NV	NV	Cluster
U	Sporadic	6006	69	P23, L5	20	Distinct
V	Sporadic	6010	121A	P13, 16, 50, L1	4	Cluster
W	Sporadic	6006	69	P23, L5	20	Distinct

\* Pipeline, rinse water from milk pipeline; Associated, epidemiologically associated human case; Sporadic, epidemiologically unrelated human case.

† P, Penner; L, Lior; NT, non-typable; NV, non-viable.

## DISCUSSION

We have described the investigations undertaken after routine testing of an unpasteurized milk supply revealed contamination with *C. jejuni*. Our investigations showed that one asymptomatic dairy cow, cow 358, was excreting *C. jejuni* directly into her milk. We also found that although faecal carriage of the organism occurred in 6 of 10 dairy cattle sampled, cow 358 did not carry *C. jejuni* in her gut, nor could it be isolated from the surface of her udder. Only when the milk from this incriminated cow was excluded from the collection, did the contamination of the bulk milk cease, suggesting that direct milk excretion was the source of the *C. jejuni* rather than faecal contamination during the milking process. As the milk pipeline rinse water was effectively a dilute sample of bulk milk, the positive *C. jejuni* culture obtained from this sample was felt to be as a result of the milk contamination, rather than a cause of it.

Strains from local cases of human *C. jejuni* enteritis associated with the consumption of this raw milk were collected for typing, along with isolates from bulk milk, cattle faeces, the milk pipeline, the individual foremilk of the

implicated cow and, for comparative purposes, epidemiologically unrelated strains.

Various methods of typing *C. jejuni* have been described, including biotyping, serotyping, phage typing, plasmid profile analysis, restriction endonuclease typing, RFLP analysis and randomly amplified polymorphic DNA profiles using PCR [20]. Many of these typing methods are time-consuming, expensive or require special equipment and expertise. PyMS is a rapid and discriminatory method for performing inter-strain comparison, which has been successfully applied to a wide range of different organisms. It may be used for initial screening of isolates from putative outbreaks in order to determine whether further study is warranted. Having determined by PyMS analysis that many of the isolates were closely related, we applied Penner and Lior serotyping, RFLP analysis, biotyping using the modified Preston scheme, and phage typing to the strains of *C. jejuni*. All methods applied clearly indicated that strains isolated from the individual foremilk of one cow, the bulk milk and two associated human cases were indistinguishable. Our study also showed that more than one strain of *C. jejuni* was carried in the gastrointestinal tract of members of the herd, and that at least one such isolate was indistinguishable from the outbreak strain. Exclusion of the cow shown to excrete the organism directly in her milk prevented any further bulk milk contamination. The results of our investigation provide evidence that direct milk excretion of *C. jejuni* by one cow was the source of at least two human cases of enteritis associated with the consumption of unpasteurized milk.

Use of the combination of typing schemes enabled us to clearly identify the outbreak strain of *C. jejuni*. In particular, strains belonging to serotype Penner 2/Lior 1 are very uncommon, and characterization of the outbreak strain using the combination of biotyping, phage typing, RFLP and Lior and Penner serotyping was therefore highly discriminatory. PyMS analysis was at variance with the standard typing methods for four isolates. Isolates A and O, although of the outbreak type, were found to be distinct by PyMS. Isolate V was of the same biotype as the outbreak strain, belonged to a different phage group, RFLP type and serotype but was indistinguishable by PyMS, and isolate Q was distinguished by biotype and phage group from the outbreak strain, but was part of the cluster by PyMS analysis. We are unable to explain these discrepancies at the present time but they may be associated with phenotypic variation.

During our investigation, we encountered some difficulties maintaining the viability of the isolates, and so were unable to submit all the original isolates to all the different typing methods. It will be important for future study of campylobacter epidemiology to have good facilities for preservation of the organism.

As a result of our investigations we have been able to convincingly demonstrate the source and route of an outbreak of *C. jejuni* enteritis. While the majority of reported cases of campylobacter gastroenteritis are so sporadic, many extensive outbreaks have been described [21–23]. Epidemiological evidence from previous studies has associated *C. jejuni* infection with the consumption of contaminated milk, usually caused by faecal contamination. Several studies have been able to demonstrate indistinguishable strains of *C. jejuni* in cattle faeces or bulk milk samples from implicated dairies, and human cases [10, 24, 25]. While direct milk



excretion of *C. jejuni* has been recognized as an alternative source of milk contamination [9, 10] this has very rarely been proved to cause human infection [2, 3, 10]. We believe that our epidemiological investigations and highly discriminatory typing results conclusively link direct udder excretion of *C. jejuni* by one asymptomatic dairy cow with cases of human gastroenteritis.

Our study highlights one of the dangers associated with the consumption of raw cow's milk. The infective dose for man of *C. jejuni* has been shown experimentally to be as low as 500 cfu/180 ml milk [26]. This is thought to be because the high fat content and fluid nature of milk protects the organism against the activity of gastric acid and leads to a relatively short gastric transit time [27]. It has frequently been observed that present technology, even with strict attention to hygiene, cannot assure the safety of raw milk [6, 18, 28, 29]. Pasteurization or other accepted form of heat treatment readily destroys campylobacter in milk and is the only practical way to render milk safe prior to retail. This investigation illustrates the need for a carefully planned approach, and the utility of the currently available typing methods to elucidate the relatedness of strains in a suspected outbreak of campylobacter.

## REFERENCES

1. Robinson DA, Jones DM. Milk-borne campylobacter infection. *BMJ* 1981; **282**: 1374–6.
2. Hudson PJ, Vogt RL, Brondum J, Patton CM. Isolation of *Campylobacter jejuni* from milk during an outbreak of campylobacteriosis. *J Infect Dis* 1984; **150**: 789.
3. Hutchinson DN, Bolton FJ, Hinchcliffe PM et al. Evidence of udder excretion of *Campylobacter jejuni* as the cause of a milk-born campylobacter outbreak. *J Hyg* 1985; **94**: 205–15.
4. Finch MJ, Blake PA. Foodborne outbreaks of campylobacteriosis: the United States experience, 1980–1982. *Am J Epidemiol* 1985; **122**: 262–8.
5. Waterman SC, Park RWA, Bramley AJ. A search for the source of *Campylobacter jejuni* in milk. *J Hyg* 1984; **92**: 333–7.
6. Humphrey TJ, Hart RJC. Campylobacter and salmonella contamination of unpasteurised cow's milk on sale to the public. *J Appl Bacteriol* 1988; **65**: 463–7.
7. Humphrey TJ, Beckett P. *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiol Infect* 1987; **98**: 263–9.
8. Lander KP, Gill KPW. Experimental infection of the bovine udder with *Campylobacter coli/jejuni*. *J Hyg* 1980; **84**: 421–8.
9. Logan EF, Neill SD, Mackie DP. Mastitis in dairy cows associated with an aerotolerant campylobacter. *Vet Rec* 1982; **110**: 229–30.
10. Morgan G, Chadwick P, Lander KP, Gill KPW. *Campylobacter jejuni* mastitis in a cow. A zoonosis-related incident. *Vet Rec* 1985; **116**: 111.
11. Morris GK, Patton CM. Campylobacter. In: Lennette EH, ed. *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, 1985; 305–6.
12. Bolton FJ, Wareing DRA, Skirrow MB, Hutchinson DN. Identification and biotyping of campylobacters. In: Board RG, Jones D, Skinner FK, eds. *Identification methods in applied and environmental microbiology*. Technical series No. 29. Blackwell Scientific Publications 1992; 151–61.
13. Freeman R, Goodfellow M, Gould FK, Hudson SJ, Lightfoot NF. Pyrolysis mass spectrometry (PyMS) for the rapid epidemiological typing of clinically significant bacterial pathogens. *J Med Microbiol* 1990; **32**: 283–6.
14. Aries RE, Gutteridge CS, Ottley TW. Evaluation of a low-cost automated pyrolysis-mass spectrometer. *J Anal Appl Pyrolysis* 1986; **9**: 81–98.
15. Ward AC, Freeman R, Sisson PR, Barer MR, Lightfoot NF. A highly discriminatory method for the direct comparison of two closely related bacterial populations by pyrolysis mass spectrometry. *Zentralb Bakt.* In press.

16. Penner JL, Hennesy JN. Passive haemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* 1980; **12**: 732–7.
17. Lior H, Woodward DL, Edgar JA. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J Clin Microbiol* 1982; **15**: 761–8.
18. Jackson CJ, Fox AJ, Jones DM. Typing of *Campylobacter jejuni* isolates by restriction fragment length polymorphisms. In: *Microbial ecology in health and disease. The VIth International Workshop in Campylobacter, Helicobacter and related organisms*. John Wiley, 1991; **4**: S53.
19. Salama SM, Bolton FJ, Hutchinson DN. Application of a new phage typing scheme to campylobacters isolated during outbreaks. *Epidemiol Infect* 1990; **104**: 405–11.
20. Mazuner S, van de Giessen A, Heuvelman K, Wernars K. Rapid analysis of campylobacter isolates; DNA fingerprinting without the need to purify DNA. *Lett Appl Microbiol* 1992; **14**: 260–2.
21. Porter IA, Reid TM. A milk-borne outbreak of campylobacter infection. *J Hyg* 1980; **84**: 415–19.
22. Jones PH, Willis AT, Robinson DA, Skirrow MB, Josephs DS. Campylobacter enteritis associated with the consumption of free school milk. *J Hyg* 1981; **87**: 155–62.
23. Potter ME, Blaser MJ, Sikes RK, Kaufmann AF, Wells JG. Human campylobacter associated with certified raw milk. *Am J Epidemiol* 1983; **117**: 475–83.
24. Robinson DA, Edgar WJ, Gibson GL, Matchett AA, Robertson L. Campylobacter enteritis associated with consumption of unpasteurised milk. *BMJ* 1979; **1**: 1171–3.
25. Kornblatt AN, Barrett T, Morris GK, Tosh FE. Epidemiologic and laboratory investigation of an outbreak of campylobacter enteritis associated with raw milk. *Am J Epidemiol* 1985; **122**: 884–9.
26. Robinson DA. Infective dose of *Campylobacter jejuni* in milk. *BMJ* 1981; **282**: 1584.
27. Potter ME, Kaufmann AF, Blake PA, Feldman RA. Unpasteurised milk. The hazards of a health fetish. *JAMA* 1984; **252**: 2050–4.
28. Skirrow MB. Foodborne illness: Campylobacter. *Lancet* 1990; **336**: 921–3.
29. Skirrow MB. Epidemiology of campylobacter enteritis. *Int J Food Microbiol* 1991; **12**: 9–16.