

Serological studies in two outbreaks of *Campylobacter jejuni* infection

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SUMMARY

Two outbreaks of campylobacter enteritis in residential student populations are described. In the first outbreak, 34 of 240 students in one hall of residence became ill over the course of 4 or 5 days. A single serotype of *C. jejuni* was isolated from samples of faeces. Sera collected demonstrated that the students had little pre-existing antibody and antibody responses were demonstrated in those who were infected. About 30% of students with serological evidence for infection with *C. jejuni* did not have gastro-intestinal symptoms.

In the second outbreak, 77 of 300 agricultural students developed symptoms over a period of 3 weeks. Unpasteurized milk was the source of infection. Two serotypes of *C. jejuni* were isolated from faeces. Antibody responses were detected to both these serotypes and to another serotype, suggesting the presence of at least 3 serotypes in the milk. Bactericidal antibody consistent with recent infection was found in all students with symptoms and in 63% of students in the absence of symptoms, indicating the possible development of immunity. The high prevalence of antibody in these students was correlated with the habitual consumption of unpasteurized milk.

INTRODUCTION

Campylobacter jejuni is now recognized as a common and important cause of diarrhoea in Britain. Much of the infection is sporadic but where outbreaks have occurred there has often been a significant association with unpasteurized or incompletely pasteurized milk (Robinson & Jones, 1981). In the outbreaks reported here we have had the opportunity to look for serological evidence of infection in each of the groups at risk and to relate this to the incidence of enteric symptoms and to previous exposure to *C. jejuni* infection.

Outbreak no. 1

In a hall of residence in Manchester 34 of 240 students became ill with abdominal symptoms, mainly diarrhoea and abdominal pain.

C. jejuni was isolated from 5 of 12 samples of faeces. The outbreak was investigated by questionnaire and serum samples from 131 students were examined.

The hall building is a tower block of 13 storeys. The students are resident and have morning and evening meals at the hall except at weekends when all meals

are available. The food is prepared in the hall kitchen, the milk supply is pasteurized but delivered in churns. There are two water supplies – a rising main supply to the lower 4 floors and a tank supply to the remainder.

The students attended a variety of different courses at a polytechnic away from the hall and apart from minor laboratory animal contact in some biological courses, and some students on catering courses, there were no potential sources of contact with campylobacter infection during the working day.

Outbreak no. 2

An outbreak of diarrhoea and abdominal pain was reported from a residential agricultural college. Between the 2 and 23 January 24 students reported to the college authorities with symptoms of gastroenteritis. *C. jejuni* was isolated from 9 of 11 samples of faeces by the local Public Health Laboratory. One student was admitted to hospital with severe abdominal pain but recovered rapidly with conservative treatment. No cases were reported from the community outside the college. An investigation was carried out using a questionnaire and a serological survey of a sample of the students.

The college has 207 students of both sexes accommodated in six halls, with a further 113 day attenders, making a total of 320 students and staff. Students take part in agricultural or horticultural courses, a few of them being involved directly with animal husbandry. Teaching takes place partly on the campus and partly in horticultural institutions and farms in the surrounding countryside. A model farm adjoining the campus is used for teaching dairy husbandry and supplies dairy products to the college. Students take all main meals on the campus and the food is prepared in central kitchens and served in one dining room. No food is provided outside mealtimes except for jugs of milk that are available to each of the six halls every evening.

Questions were asked concerning the course attended, details of any illness since the beginning of term, situation of room in the hall of residence, contact with animals and eating habits. In particular, students were asked if they took milk in tea or coffee, on cereal, or as a drink. Food for the college was purchased in bulk from local sources. Poultry was obtained fresh or frozen and stored in a cold room. Food hygiene at the college was generally of a high standard. Unpasteurized milk was obtained daily from the adjacent farm and was supplied in churns which were cleaned and sterilized at the farm. The herd was generally healthy and kept in good condition but there had been an unexpected rise in milk cell counts in the two months prior to the outbreak. Three animals in the herd had had mastitis, one of them twice during this period. Cows known to have mastitis were milked separately and the milk discarded.

Two months before this outbreak there had been a similar episode of diarrhoea and abdominal pain amongst the students but no investigations were made at that time.

MATERIALS AND METHODS

Sera were examined by a complement fixation test (CFT) using a sonicate of six different serotypes as antigen. Agglutination tests were done with suspensions of

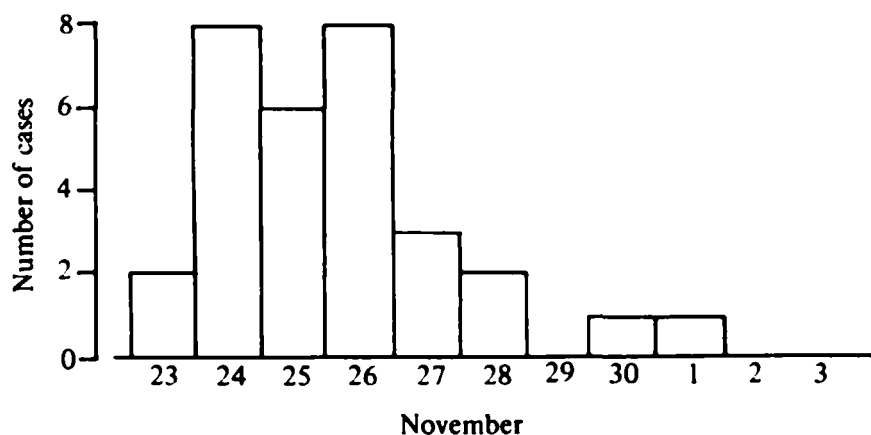


Fig. 1. Dates of onset of symptoms (outbreak 1).

C. jejuni heated to 100 °C for 15 minutes (Jones, Eldridge & Dale, 1980). Strains of *C. jejuni* isolated in each outbreak were used for detecting bactericidal antibody as follows:

·025 ml volumes of diluted inactivated serum, 1/10 dilution of fresh guinea-pig complement, and of a suspension of organisms adjusted to give semi-confluent growth on a plate, were placed in microtitre trays and incubated at 37 °C for 30 minutes. One drop from each well was then transferred to a dried blood agar plate which was incubated in N₂/CO₂ at 42 °C overnight. The end point was that dilution at which 50% of the inoculum, as compared to controls, failed to grow.

The strains of *C. jejuni* isolated from faeces were serotyped as described previously (Abbott et al. 1980).

RESULTS

Outbreak no. 1

Questionnaires were completed by 187 students; 34 mentioned abdominal symptoms. The distribution of symptoms by dates of onset is shown in Fig. 1: the average duration of symptoms was about 3 days. There was no correlation between the occurrence of symptoms and the floor of the building or room occupied. Similarly there was no correlation with the sex of the sufferer or courses attended. At the weekend, 3–4 days prior to the onset of symptoms, about half the students were absent from the hall for some meals. Those affected had no outside eating place in common. In the hall there was no particular meal that was common to all those affected, but a guest at the hall who had breakfast and lunch on the Saturday developed abdominal pains and diarrhoea 4 days later. However, a third of the students with undoubted symptoms and an accompanying antibody response did not take the Saturday lunch (roast chicken). There was a higher proportion of milk drinkers amongst those affected, 30% of this group drank milk and took it on cereal, compared to 5% of those who were not affected.

Serotyping of campylobacter strains

All five isolates were of the same serotype, provisional type 11 in our typing scheme.

Table 1. *Antibody titres to C. jejuni in 26 of 131 students (Outbreak 1)*

CFT	Agglutination	Bactericidal	Symptoms
32	160	256	+
16	40	32	+
8	20	256	+
16	320	256	+
8	320	256	+
4	40	64	+
32	80	256	+
4	20	128	+
2	40	32	+
16	< 10	256	+
4	320	256	+
4	20	256	+
4	320	256	+
8	320	256	+
32	320	256	.
16	640	128	.
8	10	256	.
2	40	256	.
32	640	< 2	.
8	160	< 2	.
32	< 10	< 2	.
4	160	< 2	.
4	40	< 2	.
4	< 10	< 2	.
4	< 10	< 2	.
4	< 10	< 2	.
total	26	21	18

Table 2. *Nature of symptoms (Outbreak 2)*

Diarrhoea and abdominal pain	28
Abdominal pain only	13
Fever, diarrhoea and abdominal pain	10
Diarrhoea only	7
Fever and diarrhoea	5
Diarrhoea, abdominal pain and vomiting	3
Diarrhoea and vomiting	2
Diarrhoea, abdominal pain, vomiting and fever	1
Fever and abdominal pain	1
Fever only	1

Serological results

Samples of blood were collected from 131 students. Twenty-six (19%) had some antibody to *C. jejuni*. 18 had specific bactericidal antibody as well as agglutinins and CF antibody, 4 had agglutinins and CF antibody, and 4 had CF antibody only. Of the 18 with bactericidal antibody, 14 had symptoms (Table 1). Of 19 students who mentioned symptoms on the questionnaire, 14 had clear serological evidence of infection (positive in all 3 tests). Of 105 students who were antibody negative, 5 recorded some mild symptoms of doubtful significance.

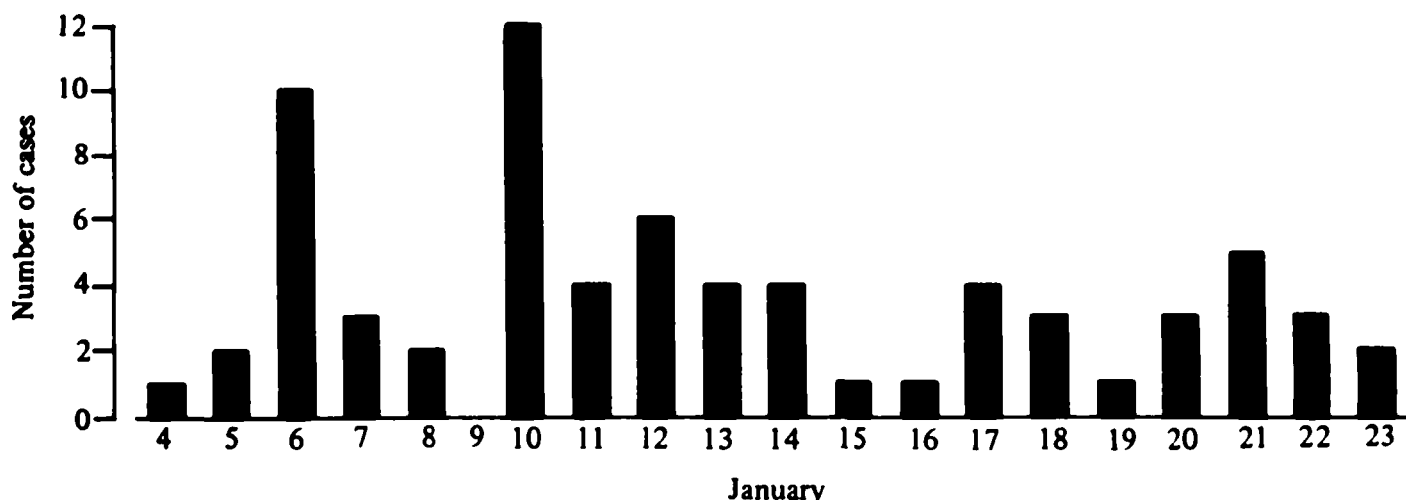


Fig. 2. Dates of onset of symptoms (outbreak 2).

Table 3. Milk consumption of 292 students (Outbreak 2)

	Symptoms	No symptoms	Total
Milk taken on cereal and as a drink	67	142	209
Milk taken in tea and coffee only	10	73	83

($\chi^2 = 12.46$, 1 D.F., $P = < 0.0001$)

Outbreak No. 2

Questionnaires were received from 292 of the 320 students and staff, 77 individuals had a history of symptoms that appeared to be relevant. The incidence of these symptoms is shown in Table 2. None had fever for more than 5 days and abdominal pain was as common as diarrhoea. The median duration of symptoms was 4 days but some complained of abdominal pain and diarrhoea lasting for 2 weeks. Distribution of cases by date of onset is shown in Fig. 2. The cases were not significantly clustered in any one hall of residence and there was no sex difference in incidence. Only eight students had had contact at work with farm animals during the term and one of these had had symptoms. Questions concerning the eating of chicken and eggs were asked and there was no correlation between the consumption of either and the development of symptoms. There was a significant difference between the quantity of milk consumed by individuals in the symptomatic and the asymptomatic groups (Table 3).

Serotyping of Campylobacter strains

There were two serotypes among seven strains isolated from faeces (Table 4). Strains 1572 (type 2/5) and 1570 (type 6) were used for bactericidal tests on the patients' sera.

Serological results

Complement fixing antibodies. Thirty eight sera (80%) had complement fixing antibody present to a titre of 1/4 or greater. All 15 patients with symptoms in

Table 4. *Serotyping reactions of 7 strains of C. jejuni (Outbreak 2)*

Strain	Serotype						
	1	2	3	4	5	6	7
1568	.	+	.	.	+	.	.
1569	+	.
1570	+	.
1571	.	+	.	.	+	.	.
1572	.	+	.	.	+	.	.
1574	.	+	.	.	+	.	.
1575	.	+	.	.	+	.	.

Table 5. *Complement fixing antibody in 37 students (Outbreak 2)*

	Reciprocal titre								Total
	≤ 2	4	8	16	32	64	128	256	
Recent symptoms	0	2	2	3	3	2	2	1	15
No recent symptoms	10	3	5	6	3	5	0	0	32

Table 6. *Agglutinins to heated suspensions of C. jejuni 1572 (Outbreak 2)*

	Reciprocal titre						Total
	< 80	80	160	320	640	1280	
Recent symptoms	4	2	2	4	1	2	15
No recent symptoms	16	2	2	8	4	0	32

Table 7. *Bactericidal antibodies to C. jejuni 1572 (Outbreak 2)*

	Reciprocal titre							Total
	< 4	4	8	16	32	64	≥ 128	
Recent symptoms	4	1	2	1	2	0	5	15
No recent symptoms	12	2	0	2	3	2	11	32

the sample were CF positive. Of the 32 students who did not have recent symptoms, 22 (69%), had CF antibody (Table 5).

Agglutinins. Twenty seven sera (57%) had agglutinins to strain 1572 at a titre of 1/80 or greater (Table 6). Agglutinations with strain 1570 gave somewhat similar results and these were unrelated to the presence of bactericidal antibody to 1570. This lack of specificity made agglutination results difficult to interpret except that they indicated past infection with some serotype of *C. jejuni*.

Bactericidal antibodies. Thirty one sera (66%) were bactericidal to strain 1572, whereas only four sera (8.5%) were bactericidal to strain 1570, and these four sera were also bactericidal to 1572. The bactericidal titres of those students with and without symptoms is shown in Table 7.

Although nine of the students tested had neither bactericidal nor CF antibody and so presumably had not had a recent infection, seven students had CF antibody

Table 8. Complement fixing antibodies in individuals not having bactericidal antibody to strains 1572 and 1570 (Outbreak 2)

	Reciprocal titre						Total	
	≤2	4	8	16	32	64		128
Recent symptoms	0	0	1*	1*	2	0	0	4
No recent symptoms	9	0	2	0	0	1*	0	12

* These students had bactericidal antibody to *C. jejuni* serotype 9.

in the absence of bactericidal antibody to the two strains used (Table 8), and four of these had had recent symptoms. The sera from these students were tested for bactericidal antibody to 10 different serotypes that were included in our typing system. Three had bactericidal antibody to serotype 9.

DISCUSSION

In outbreak no. 1 the onset of symptoms occurred over just a few days and were presumably the result of exposure to infection for only a short time. The strains of *C. jejuni* that were isolated were all of the same serotype. No single meal was common to those affected and, although it could not be proved, it seemed possible that contaminated milk from a single churn may have been the vehicle.

Following infection with *C. jejuni*, antibody appears that can usually be detected by all three methods used in this study, although occasional individuals will be negative in one test. The bactericidal test is serotype-specific but agglutination and particularly complement-fixation have a broader specificity. Of 18 students who had an unequivocal antibody response in all three tests, four did not have symptoms. This finding suggests that at least 20–25% of *C. jejuni* infections may be symptomless (Table 1).

The students who had only CF antibody, or had CF antibody with low levels of agglutinins, probably represented the general 'background' of antibody to *C. jejuni* in the general population. We have previously found that between 2 and 5% of antenatal patients have complement fixing antibody to *C. jejuni* (Jones & Robinson, 1981).

In outbreak no. 2 the nature of the symptoms, serological data and the isolation of *C. jejuni* confirmed that this was also an outbreak of gastroenteritis due to *C. jejuni*. Residence in particular halls, consumption of particular foods, or animal contact, were not correlated with infection. There was a correlation between the incidence of symptoms and the amount of unpasteurized milk consumed by the students. The spread of the dates of onset over nearly a month was consistent with the presence of *C. jejuni* in the milk on numerous occasions. The isolation of two different serotypes from faeces and serological evidence for infection with a third serotype indicates that the milk was contaminated with different strains of *C. jejuni*. These may or may not have been in the milk at the same time. Serological response to two serotypes simultaneously in 4 students suggests that these may have had double infections, and as not all students had a similar response it is possible that one strain (type 2/5) may have been present in milk in larger numbers than the other (type 6).

It is likely that these students had been repeatedly exposed to *C. jejuni* infection in view of the high prevalence of CF antibody, present in all of those tested with symptoms and 69% of those without. Bactericidal antibody to serotype 2/5, indicating recent infection with this serotype, was also present in 20 out of 32 (63%) students in the absence of symptoms.

This proportion of students with evidence of recent infection and no symptoms is higher than in outbreak no. 1 (20%) and it may represent an immunity from gastro-intestinal symptoms in individuals being constantly reinfected with *C. jejuni*. Some students with no bactericidal antibody had high titres of CF antibody in keeping with recent infection (Table 7). While some of these individuals may belong to the minority that do not develop bactericidal antibody after infection, it was thought that they may have been infected with serotypes other than the two grown from the few samples of faeces that were cultured. Screening their sera with just 10 serotypes of *C. jejuni* showed that three students had bactericidal antibody to *C. jejuni* type 9, substantiating the idea that other serotypes had been present in the milk.

Agglutination results were found not to be type-specific and in a group of individuals with past or repeated *C. jejuni* infection the agglutination response and its interpretation was uncertain. In each outbreak the presence of agglutinating antibody indicated previous infection but not as effectively as the CF test. The prevalence of CF antibody was seen to be a good index of previous exposure to *C. jejuni* infection; demonstrating a group with a 'normal' background in outbreak no. 1 and a group with a very different previous experience of *C. jejuni* in outbreak no. 2. It is likely that the main reason for this difference between the two populations was that the second group of students were habitual drinkers of unpasteurized milk.

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