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Application of three typing schemes (Penner, Lior, Preston) to strains of *Campylobacter* spp. isolated from three outbreaks

By D. N. HUTCHINSON*, F. J. BOLTON

The Public Health Laboratory, Preston Infirmary, Meadow Street, Preston, Lancashire, PR1 6PS, UK

D. M. JONES, E. M. SUTCLIFFE and J. D. ABBOTT

The Public Health Laboratory, Withington Hospital, Manchester, M20 8LR, UK

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SUMMARY

Campylobacters isolated from human, animal and environmental sources during the investigation of a milk-borne outbreak, an incident involving goats' milk and an outbreak associated with puppies were serotyped using the Penner and Lior schemes and biotyped using the Preston scheme. Application of these three methods to the incident strains demonstrated that heterogenicity amongst similar strains exists. This study has confirmed the need to use at least two typing methods when investigating epidemiologically-related strains and we suggest that a combination of a serotyping scheme and an extended biotyping scheme is the most useful.

INTRODUCTION

Outbreaks of campylobacter enteritis associated with the consumption of raw milk, water and other foods have been reported and reviewed by Blaser, Taylor & Feldman (1985). To determine the origin of outbreaks it is necessary to use typing methods which are discriminatory. The haemagglutination method of Penner & Hennessy (1980) and the slide agglutination method of Lior *et al.* (1982) are both widely used for serotyping strains of *Campylobacter* species. The value of serotyping strains from outbreaks using two different serotyping methods has been reported (Jones, Sutcliffe & Abbott, 1985). An alternative to serotyping is biotyping and the Preston scheme has been used for the epidemiological typing of strains from outbreaks (Bolton, Holt & Hutchinson, 1984).

The advantages of applying combinations of serological and biotyping schemes has therefore been assessed with strains from three outbreaks. These were, an extensive outbreak of campylobacter enteritis associated with untreated milk (Hutchinson *et al.* 1985*a*), an incident associated with consumption of goats' milk (Hutchinson *et al.* 1985*b*) and an outbreak associated with dogs. This latter outbreak originated from a litter of 11 puppies which were given to local residents of a neighbourhood community. All but one of the puppies died with enteritis and most of the households which received puppies had human cases. A total of 9

* Correspondence to Dr D. N. Hutchinson.

households were infected and 16 human cases diagnosed. One of these households also contained two adult dogs which had become asymptomatic campylobacter excreters (details of the outbreak to be published elsewhere). The three outbreaks were selected for study because of the diversity of sources from which campylobacters were isolated. Strains from human cases, associated animals and the environment were examined using the serotyping schemes of Penner & Hennessy (1980) and Lior *et al.* (1982) and the Preston biotyping scheme (Bolton, Holt & Hutchinson, 1984).

MATERIALS AND METHODS

Organisms

These included thermophilic campylobacters isolated during the epidemiological investigations of the three outbreaks. Strains were kept at −70 °C in Brain Heart Infustion broth (Difco) containing 20 % glycerol until required for typing.

Serotyping

The two methods used were the passive haemagglutination technique of Penner & Hennessy (1980) and the slide agglutination technique of Lior *et al.* (1982). Both serotyping procedures were performed as described previously by Jones, Sutcliffe & Abbott, (1985).

Biotyping

Isolations were biotyped as described previously (Bolton, Holt & Hutchinson, 1984) except that the concentration of safranin-O was increased to 0·1 % and that of triphenyl tetrazolium chloride (TTC) to 0·1 %.

RESULTS

The results of typing of the 98 *C. jejuni* strains isolated from the milk-borne outbreak are shown in Table 1. The outbreak strain was *C. jejuni* Preston biotype code 6100, Penner serotype 50 (including cross-reacting types 4, 13, 16) and Lior serotype 7 (including cross reactions with types 1 and 2). All the milk isolates and all but one of the human isolates were of the outbreak 'type'. The only non-outbreak strain isolated from a human was recovered from an asymptomatic farm worker. Several of the cattle midden and water isolates were also of the outbreak 'type' but the remaining environmental isolates were of various biotypes and serotypes.

Table 2 presents the results of typing 24 strains isolated from the goats' milk incident. The strain responsible for this incident was *C. coli* Preston biotype code 2314, Penner serotype 49 but Lior non-typable. This strain was isolated from only one human case, but similar strains were isolated from three goats and a sample of poultry faeces collected at the implicated farm. Of the other strains isolated from specimens collected at the farm, only one was *C. coli* but this was a different biotype than the incident strain and it was also non-typable by both serotyping schemes. All other isolates were *C. jejuni* of various biotypes and Penner and Lior serotypes.

The results of typing 22 of the strains from the canine associated outbreak are shown in Table 3. Two strains were isolated from infected human cases. The main

Table 1. *Serotypes and biotypes of 98* C. jejuni *strains from a milk-borne outbreak of campylobacter enteritis*

Strain designation			Source of isolates					
Preston biotype code	Penner serotype	Lior serotype	Human faeces	Cattle rectal swabs	Milk*	Midden	Sheep faeces	Surface water
Outbreak strains								
6100	50	7	34	2	2	—	—	—
6100	50	1, 7	4	—	—	—	—	—
6100	50	2, 7	7	—	—	—	—	—
6100	50	1, 2, 7	1	—	—	—	—	—
6100	50	2	1	—	—	1	—	—
6100	50	NT	—	2	—	—	—	—
6100	13, 50	7	4	2	4	2	—	—
6100	13, 50	2, 7	2	—	—	—	—	—
6100	13, 16, 50	7	1	—	1	—	—	—
6100	16, 50	7	2	—	—	—	—	1
6100	4, 16, 50	7	—	—	1	—	—	—
Other strains isolated during the investigation								
6100	1	2	—	1	—	1	—	—
6112	2	4	—	1	—	—	—	—
6320	3	NT	1	—	—	—	—	—
6100	4	NT	—	2	—	—	—	—
6350	6, 7	6	—	—	—	—	—	1
6100	8	1	—	—	—	—	1	—
6000	23	5	—	1	—	1	—	—
6100	23	5	—	5	—	2	—	—
6100	23	7	—	—	—	—	3	—
6350	40	4	—	—	—	2	—	—
6300	48	NT	—	1	—	—	—	—
6300	NT	NT	—	1	—	—	—	—

* Includes isolates from bulk milk, milk filters, retailed bottled milk.
NT, Not typable.

outbreak strain was *C. jejuni* Preston biotype code 6100, Penner serotype 4 (including minor reactions with 13, 50) and Lior serotype 1. This strain was isolated from 10 human cases, 2 puppies and 2 adult dogs. Four other human cases were infected with a similar but not identical strain which was *C. jejuni*, Preston biotype 6100, Penner serotype 13, 50 and Lior serotype 1. One other isolate from a puppy was similar to the latter strain but was non-typable by the Lior method. Three of the four households with cases infected with the second strain had either other human cases or an infected puppy excreting the main outbreak strain. Of the remaining strains one human case was infected with a non-outbreak strain (*C. jejuni* Preston biotype 6154, Penner serotype 15 and Lior non-typable) and may have been a coincidental finding. Another case, a child, was infected with a different strain of *C. jejuni* (Preston biotype code 6350, Penner serotype 40 and Lior non-typable). This strain was also isolated from an adult dog which belonged to a different household but which was frequently visited by the infected child and her mother.

Table 2. *Serotypes and biotypes of 24 campylobacter isolates from an incident of campylobacter enteritis associated with the consumption of raw goats milk*

Strain designation			Source of isolates					
Preston biotype code	Penner serotype	Lior serotype	Human faeces	Goat rectal swabs	Bulk milk	Individual milk	Midden	Poultry faeces
Incident strain								
C. coli								
2314	49	NT	1	3	—	—	—	1
Other strains isolated during the investigation								
C. coli								
2114	NT	NT	—	—	—	—	—	1
C. jejuni								
6052	31	NT	—	1	—	—	—	—
6100	2	NT	—	1	—	—	—	—
6110	15	NT	—	1	3	—	1	—
6150	40	11	—	—	—	—	—	1
6150	NT	11	—	1	—	—	—	—
6150	NT	NT	—	—	—	—	—	1
6152	49	2	—	1	—	—	—	—
6152	49	4	—	1	—	—	—	—
6152	49	NT	—	1*	1*	—	—	—
6152	NT	NT	—	1	—	—	—	—
6154	NT	NT	—	—	—	—	—	2

* These two strains were isolated from different goats.
NT, non-typable.

Table 3. *Serotypes and biotypes of 22* C. jejuni *strains from an outbreak of campylobacter enteritis transmitted by dogs*

Strain designation			Source of isolates	
Preston biotype code	Penner serotype	Lior serotype	Human faeces	Canine faeces
Main outbreak strain				
6100	4 (13, 50)*	1	10	4
Other outbreak strains				
6100	13, 50	1	4	—
6100	50	NT	—	1
Other strains isolated during the investigation				
6254	15	NT	1	—
6350	40	NT	1	1

* Several strains possessed antigens 13 and/or 50.
NT, Not-typable.

DISCUSSION

Application of the three typing schemes to strains from the milk-borne outbreak (Table 1) demonstrated that the outbreak strain was more clearly recognized by Penner serotyping and that use of biotyping or Lior serotyping alone would have been less definitive. Initially, outbreak strains were differentiated from non-outbreak strains by the Penner serotyping scheme. The outbreak strains belonged

to serotype 50, with 4, 13, 16 expressed to varying degrees which could confuse the epidemiological picture unless another typing method was performed concurrently. Similarly, the Lior serotype 7 strains showed some cross-reaction with serotypes 1 and 2 and these three antigens were present in varying combinations in the outbreak strains. Interestingly, two of the isolates from cattle, included as outbreak strains, were non-typable by the Lior method. These strains may have lost the 1, 2 or 7 antigenic determinants due to loss of motility and further subculturing may have been needed for antigenic expression. Alternatively testing with a wider range of Lior antisera may have revealed the presence of other antigens. Lior serotyping alone would not have clearly defined the extent and possible origins of the incident because antigens 1, 2 and 7 were present in six of the non-outbreak strains. All of the outbreak strains were *C. jejuni* of Preston biotype code 6100 but if this method had been used alone it would not have been sufficiently discriminatory because 13 out of 22 of the non-outbreak strains also gave this code.

In the second incident involving goats' milk serotyping of isolates by either method alone would not have yielded sufficient epidemiological information and may have been inconclusive. The incident strain was *C. coli* Penner serotype 49 but three *C. jejuni* isolates were also of this serotype. Lior serotyping was not helpful in this outbreak because many of the isolates were non-typable probably for the reasons mentioned previously. Since *C. coli* strains are responsible for about 5% of cases of campylobacter enteritis in the United Kingdom (Skirrow & Benjamin, 1982) the isolation of such a strain from a case and environmentally-related specimens is highly suggestive of an epidemiological association. Nevertheless both serotyping and biotyping should be used in conjunction.

In the third outbreak in which transmission of infection was associated with infected puppies and dogs, any two methods used in combination would have produced adequate epidemiological information. Interestingly, if only the Preston biotyping scheme and the Lior serotyping scheme had been used then 14 cases and 4 canines would have been considered to be infected with the same strain. However, Penner serotyping exhibited heterogenicity amongst these strains which was related to the detection of the type 4 antigen and this produced division of the outbreak strains. It is possible that some strains may have lost the major type 4 antigen and therefore all of the outbreak stains would be related.

The application of the three typing schemes has demonstrated that heterogenicity amongst apparently similar strains exists. Strains which are of similar serotypes by both Penner and Lior schemes may be of different biotypes, or strains of the same biotype may all be of the same serotype by one serotyping scheme but of different serotypes by a second scheme. Hence, investigation of these three episodes has highlighted the problems associated with the application of a single typing method tō the epidemiological study of outbreaks or incidents when human, animal and environmental isolates are involved. Whilst one scheme may suggest that strains are identical, the use of a second or third scheme is necessary for confirmation. We suggest that at least two typing schemes should always be used, preferably a serotyping scheme and an extended biotyping scheme because strains would always be typable by the latter method but may not be typable by serological methods.

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