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Application of a new phagetyping scheme to campylobacters isolated during outbreaks

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SUMMARY

A new scheme for phagetyping campylobacters has been evaluated using strains isolated from five outbreaks. The phagetyping results have been compared with the results of Penner serotyping, Lior serotyping and Preston biotyping. Phagetyping recognized the causative strains in all of the incidents and also differentiated these strains from animal and environmental strains isolated during these investigations. In some outbreaks phagetyping proved to be more discriminatory than serotyping or biotyping, e.g. strains of Penner serotype 2, and serogroup 4, 13, 16, 50 were subdivided by this method. Phagetyping is to be recommended for typing strains from outbreaks and although the results indicate that it may be used alone we advocate that it should be used in conjunction with one of the established typing methods.

INTRODUCTION

Thermophilic campylobacters (*Campylobacter jejuni* and *C. coli*) are the commonest bacterial cause of enteric infections in developed countries. To elucidate the sources and modes of transmission of these organisms it is essential to use epidemiological typing methods which discriminate between different strains but which reliably and reproducibly recognize similar strains. Campylobacters can be typed by biotyping [1–3], serotyping [4, 5], plasmid typing [6–8], enzyme profile analysis [9], restriction digest analysis [10] and a phagetyping scheme has been described by Grajewski and colleagues [11] in the USA. This scheme has undergone further evaluation by workers in Canada [12] who suggested that the scheme needed to be improved. We have previously reported the isolation of campylobacter bacteriophages from a variety of sources [13] and have combined ten of these phages with six of those isolated by Grajewski and co-workers [11] to form a new phagetyping scheme.

To determine the epidemiological value of the new phagetyping scheme, we have tested strains isolated from several outbreaks and which have been biotyped and serotyped.

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MATERIALS AND METHODS

Source of strains

The strains tested were isolated from five outbreaks. Two of these were associated with schools; the outbreak in school A was probably caused by school milk whereas the outbreak in school B was associated with contamination of the school private drinking water supply. Details of the other outbreaks have been reported previously, i.e. puppy outbreak [14], milk-borne outbreak [15] and the goat's milk incident [16]. Strains from the outbreak at school B were recent isolates whereas all other strains had been stored at −70 °C in brain heart infusion broth containing 20% glycerol.

Phagetyping method

Phages were propagated and titrated to determine the routine test dilution (RTD) using conventional techniques [17]. Test strains were subcultured onto blood agar plates, incubated microaerobically at 42 °C for 24 h and the growth harvested into Brain Heart Infusion broth (Difco) to give suspensions with an optical density of 2·0 at 420 nm (approximately 10^8 colony forming units (c.f.u./ml) with a Perkin Elmer 6/20 spectrophotometer. These suspensions were flooded onto the dried surface of plates of Brucella agar (Oxoid CM691) and excess fluid discarded. Inoculated plates were allowed to dry at room temperature and then inoculated with 10 μl of each of the 16 phage suspensions at RTD using the Lidwell phage applicator. Plates were incubated microaerobically at 42 °C for 18–24 h and examined for confluent lysis or plaques. Lysis was recorded as + + + if confluent or semi-confluent, + + if 50–100 plaques, + if 20–49 plaques and ± if 1–19 plaques. Only phage reactions of + + + or + + were recorded as positive. Phage patterns were recorded and assigned to one of the previously designated phage groups. These were defined from studies using more than 1000 sporadic isolates from different parts of the UK. Phagetypes with similar lytic patterns, i.e. two or fewer differences, have been clustered to form the phage groups. There are currently 140 phage groups (details to be published).

RESULTS

The phagetyping, biotyping and serotyping results of campylobacters isolated during the two school outbreaks are shown in Table 1. In the outbreak at school A phagetyping and biotyping gave concordant results. Phagetyping clearly recognized the two causative strains (phage groups 52 and 59) whereas the serotyping results with the major outbreak strain (phage group 52) were less clear. In the outbreak at school B biotyping and serotyping gave concordant results but phagetyping was more discriminatory and differentiated the strains into three phage groups (40, 37, 94).

The results of typing strains from the puppy associated outbreak are summarized in Table 2. The causative strain was recognized by all four typing methods and strains which exhibited the various antigens of the Penner 4, 13, 50 serogroup complex were recognized as the same by phagetyping. Two other strains

Table 1. *Typing of campylobacters isolated from outbreaks in schools*

School	Year	Provisional phage-group	Preston biotype	Serotype		Number of strains isolated from humans
				Penner	Lior	
A	1987	52	6100	2	6	12
		52	6100	2	17	3
		52	6100	2	NT	1
		52	6100	NT	NT	1
		59	6112	19	NT	3
B	1988	40	6102	1	12	9
		37	6102	1	12	2
		94	6102	1	2	1

NT, not typable.

Table 2. *Typing of campylobacters isolated during an outbreak transmitted by puppies.*

Provisional phage-group	Preston biotype	Serotype		Number of strains isolated from	
		Penner	Lior	Humans	Dogs
86	6100	4, 13, 50*	1	11	4
59	6154	15	NT	1	1

* Includes strains expressing any combination of these antigens. NT, not typable.

which were epidemiologically linked, one from a human, the other from an adult dog, were shown to be identical by phagetyping, biotyping and Penner serotyping.

The results of typing strains from the milk-borne outbreak are presented in Table 3. The causative strain isolated from cases, milk and cattle was recognized by all of the typing methods. Isolates of phage group 44 (causative strain) were all of the same biotype but exhibited the Penner serogroup 4, 13, 16, 50 antigens and the Lior 2 and 7 antigens to different degrees. Phagetyping and Penner serotyping clearly differentiated between the causative and non-causative strains whereas some of the latter strains were of the same biotype and Lior serotype as the causative strains.

The incident associated with goat's milk (Table 4) was caused by a strain of *C. coli* which was readily identified by biotyping and clearly differentiated from other animal and environmental strains by phagetyping. Penner serotyping recognized the *C. coli* strain as serotype 49 and also a *C. jejuni* as the same serotype. This latter strain was shown to be different from the *C. coli* by phagetyping, biotyping and Lior serotyping.

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Table 3. *Typing of human, animal and environmental campylobacter strains isolated during investigation of a milk-borne outbreak*

Provisional phage-group	Preston biotype	Serotype		Number of strains isolated from					
		Penner	Lior	Human	Milk	Cattle	Sheep	Midden	Water
Causative strain									
44	6100	4, 13, 16, 50*	2, 7*	27	2	5	0	0	1
Non-causative strains									
59	6100	23	5	0	0	5	0	2	0
37	6102	2	1	0	3	0	0	0	0
37	6112	2	4	0	0	1	0	0	0
37	6100	8	1	0	0	0	1	0	0
31	6350	40	4	0	0	0	0	2	0
77	6100	23	7	0	0	0	3	0	0

* Includes strains expressing any combination of these antigens.

Table 4. *Typing of campylobacters isolated during an incident associated with goat's milk*

Provisional phage-group	Preston biotype	Serotype		Number of strains isolated from				
		Penner	Lior	Human	Goat rectal swab	Goat's milk	Midden	Poultry
Causative strain								
59	2314	49	NT	1	3	0	0	1
Non-causative strain								
105	6052	49	4	0	1	0	0	0
3	6100	2	NT	0	1	0	0	0
3	6152	NT	NT	0	1	0	0	0
106	6150	NT	11	0	0	0	0	2
NT	6110	15, 46	NT	0	0	3	2	0

NT, not typable.

DISCUSSION

Organisms isolated during the investigation of outbreaks should be typed by more than one method to confirm that isolates are identical. In a previous study [18] using campylobacters isolated from outbreaks we evaluated a biotyping scheme [3] and two serotyping schemes [4, 5] and concluded that more than one method was necessary for typing these organisms. We proposed that a serotyping scheme used in conjunction with a biotyping scheme was most appropriate.

In the present study we have evaluated a new phagetyping scheme which successfully recognized the causative strain in each of the five outbreaks. Moreover, this scheme differentiated the causative strains from other animal and environmental strains isolated during these investigations. The phagetyping scheme typed all of the human and most of the animal and environmental isolates, which highlights the potential value of the scheme for studying the epidemiology of *Campylobacter* infections. A successful scheme should also be capable of typing the majority of strains associated with sporadic infections and this study is currently being completed. In the present study many of the isolates had been kept frozen but this did not affect the ability of the strains to be typed by the panel of phages which indicates that the surface receptors for the phages are stable. This has been confirmed in other studies in which we repeatedly tested strains which had been stored and obtained the same phagetyping results (unpublished observations).

Serotyping of stored strains by the Lior method can be problematical, strains may have to be passaged numerous times and even then may not produce a serotype. The phagetyping scheme, therefore has an advantage when examining stored strains. Reliance on the results of serotyping alone can be misleading and confusing. Strains of Penner serogroup complex 4, 13, 16, 50 can express these antigens to different degrees and this may lead to confusion about the similarity of such isolates. In the two outbreaks associated with this serogroup complex (Tables 2 and 3) phagetyping clarified the problem of antigenic variation and confirmed that the isolates from cases in each of the outbreaks were the same. In our previous study [18] Lior serotyping did not help to resolve this problem because some strains were non-typable either because of the storage problem or because only a limited number of antisera were employed. It is therefore difficult to comment on the value of combining the results of Lior serotyping with phagetyping.

There is also some evidence from the present study that phagetyping can subtype strains of the same serogroup or serotype. This is shown by the results with the Penner serogroup 4, 13, 16, 50 strains from the puppy outbreak (Table 2) and the milk-borne outbreak (Table 3) which were caused by phagegroups 86 and 44 respectively. The Penner serogroup 4, 13, 16, 50 is common amongst human sporadic isolates and accounted for 21% of strains tested during a study undertaken in the north of England [19]. The ability to subdivide this serogroup would be analogous to phagetyping of *Salmonella typhimurium* isolates and would hopefully lead to clearer epidemiological information.

Conversely it can be seen that an alternative typing scheme will distinguish between strains of the same phage group, e.g. strains of phage group 39 were

isolated from three different outbreaks and proved to be different Penner serotypes. The phagetyping scheme is therefore complementary to Penner serotyping.

In the present study phagetyping clearly differentiated causative strains from non-causative strains which would indicate that the scheme is useful for the initial screening of strains from outbreaks. We would, however, recommend that the phagetyping scheme be used in conjunction with another typing method. Furthermore phagetyping is a useful alternative for laboratories, which cannot use serotyping.

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