JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1982, p. 281–285
0095-1137/82/080281-05$02.00/0

Vol. 16, No. 2

Serotyping of *Campylobacter jejuni* Isolated from Sporadic Cases and Outbreaks in British Columbia

P. M. S. McMYNE,[1]* J. L. PENNER,[2] R. G. MATHIAS,[1] W. A. BLACK,[1] AND J. N. HENNESSY[2]

Division of Laboratories, Ministry of Health, Vancouver, British Columbia, Canada V6J 4M3[1]; and Department of Medical Microbiology, Faculty of Medicine, University of Toronto, Banting Institute, Toronto, Ontario, Canada M5G 1L5[2]

Received 3 February 1982/Accepted 12 May 1982

Campylobacter jejuni from sporadic cases and outbreaks of gastroenteritis were serotyped on the basis of heat-extracted soluble thermostable antigens identified with the use of the passive hemagglutination technique. A total of 168 isolates were separated into 45 different types. The largest proportion of the isolates fell into three serotypes, each with 11 to 12.5% of the total number. Three less frequently occurring serotypes each included approximately 5%, and the remaining 50% of the isolates were distributed among 39 other serotypes. In most cases, serotyping demonstrated that epidemiologically linked isolates were of the same serotype, but the outbreak strains could belong either to frequently or to infrequently isolated serotypes. The high correlation between clinical findings and serotyping results confirmed the applicability of the serotyping scheme in epidemiological investigations of *C. jejuni* infections.

In the last 10 years, it has been well documented that *Campylobacter jejuni* bacteria are a common cause of enteritis in humans (4–6, 8, 13, 20, 23). Methods have been developed and widely adopted for routine isolation of these agents (6, 8, 12, 23), but much still remains to be learned about the epidemiology of the infections.

Previous studies have drawn attention to the possible transmission of the infectious agents through contaminated water, milk, or food (10, 11, 18, 19, 22, 25). Some indirect evidence suggests that household pets or domestic animals may be the source of human infections (2, 3, 24). To confirm these suspected routes of sources and transmissions, effective discrimination between strains of the species is required. Progress in this area has been limited. Since the bacteria have only a few biochemical reactions that may be routinely demonstrable in the clinical laboratory (6, 12, 16), it is not expected that biotyping will be adequate for epidemiological studies. Interest has therefore focused on the use of serotyping methods for epidemiological tracing. Several different serotyping schemes have already been described, but all are still in the developmental stages (1, 14, 15, 17, 21). Adoption by public health laboratories and reference centers of one scheme or another will depend to a large extent upon the effectiveness of the scheme in the practical situation.

In this communication, we describe a study in which one such scheme was used to serotype isolates submitted to a public health laboratory with the objectives of assessing the scheme for its performance in epidemiological studies and for gaining new insights into the epidemiology of infections caused by *C. jejuni*.

MATERIALS AND METHODS

Isolation and identification of bacteria. Fecal specimens in Cary-Blair transport medium (7) from different areas in the province were sent to the British Columbia Provincial Health Laboratory for isolation and identification of diarrhea-producing pathogens. For isolation of *C. jejuni*, each specimen was inoculated onto Columbia agar base medium (Oxoid) supplemented with 7% horse blood and antibiotics (SR 85; Oxoid). The plates were incubated at 43°C for 48 h in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Colonies that were gram negative with curved or S-shaped morphology, positive in tests for oxidase and catalase production, and susceptible to nalidixic acid (30 μg/ml) were classified as *C. jejuni*.

Sources of strains to be serotyped. Strains fell into three categories: (i) strains from an outbreak of gastroenteritis at Nakusp, British Columbia; (ii) strains from seven families; and (iii) strains from sporadic cases.

Serotyping. Subcultures of *C. jejuni* were sent in Amies transport medium (Difco Laboratories) to the laboratory of one of us (J.L.P.) for serotyping. The swab from the transport medium was first used to inoculate 5 ml of thioglycolate broth (BBL Microbiology Systems) and then a blood agar plate (Columbia agar with 5% whole horse blood). The inoculated media were incubated at 37°C in a CO_2 incubator (Forma Scientific, Marietta, Ohio) set to maintain an atmosphere of 5% CO_2. This atmosphere, unsuitable for primary isolation, readily supported the growth of strains submitted for serotyping. After 48 h, each plate was checked for purity and then transferred to and

TABLE 1. Community outbreak at Nakusp, British Columbia, Canada

Patient	Age (yr)	Date specimen received	Serotype	Titer[a]
1	27	9/7/80	1	1:5,120
2	20	11/7/80	1	1:5,120
3	21	14/7/80	1	1:5,120
4	56	23/7/80	1	1:2,560
5	39	30/7/80	1	1:2,560
6	59	30/7/80	1	1:640
7	25	17/7/80	5	1:5,120
	25	29/7/80	5	1:2,560

[a] Titers are for antisera against the indicated serotype. Reactions in antisera against all other serotypes were ≤40.

spread over the surface of four blood agar plates. After incubation for 48 h at 37°C, 1.5 ml of saline (0.85% NaCl) was added to each plate, and bacteria were scraped from the four plates and pooled in screw-capped tubes. The tubes were heated for 1 h in boiling water. After cooling and centrifugation (8,000 × *g* for 10 min), the supernatant fluid was removed and used to sensitize sheep erythrocytes for passive hemagglutination.

If pure cultures were not seen on first transfer, the swab from the transport medium (maintained at 4°C in the interval) was used to inoculate a plate of Columbia agar base (Oxoid) containing 7% horse blood and antibiotics according to the formula of Skirrow (23) and incubated for 48 h at 37°C in the CO_2 incubator. If Gram stains revealed the typical morphology of *C. jejuni*, colonies were selected to inoculate blood agar plates as above. Growth from thioglycolate broth was used to inoculate Skirrow medium when subculturing from transport medium failed to yield *C. jejuni*.

Passive hemagglutination was performed as described previously (21). Each antigen preparation was titrated against 55 antisera prepared against stock serotype strains. Titrations were performed in microtitration plates. Antisera were serially twofold diluted in 11 wells. Another well without antiserum contained only diluent. Suspensions of antigen-coated sheep erythrocytes were added to and mixed with the contents of each well. After 1 h of incubation at 37°C and overnight storage at 4°C, the highest dilution in which agglutination was observed was taken as the titer. No agglutination of sensitized erythrocytes was observed in the well that served as a control. Each isolate was then assigned a serotype according to the antiserum (or antisera) in which agglutination occurred. Antisera were not absorbed. In instances of multiple displays of antigens, the titers obtained were compared with those of known cross-reactions of standard strains to identify the serotype(s) present. This procedure was undertaken to detect as many combinations of antigens on unknown strains as possible.

RESULTS

Retrospective serotyping of isolates from patients of a community outbreak of gastroenteritis. Nine strains were obtained from eight patients at Nakusp, British Columbia, where an outbreak of gastroenteritis was reported (9). Six isolates reacted only in antiserum against serotype 1 and titers ranged from 1:640 to 1:5,120 (Table 1). No significant reactions were seen with the other 54 antisera. Three isolates, two from one individual and a third from another, reacted only in antiserum against serotype 5. The third isolate was obtained 1 month after the outbreak and was therefore considered not to have been associated with the outbreak; thus, it is not included in Table 1.

Those strains belonging to serotype 1 were positive in tests for hippurate hydrolysis and were susceptible to 10 μg of ampicillin per ml. The two serotype 5 isolates were hippurate negative and were not inhibited by concentrations of the antibiotic less than 40 μg/ml.

Having determined that this typing method discriminated between isolates of the species and identified the common strain involved in this outbreak, we arranged a program for continuous serotyping between the laboratories at Vancouver and Toronto.

Serotypes of isolates from family outbreaks. Isolates from seven suspected family outbreaks are shown in Table 2. It can be seen that in all but one outbreak, each family member or animal contact had an isolate of the same serotype as others involved in the particular outbreak. The serotype of the outbreak strains were different from one family to the next. They included some very common serotypes (1, 2, and 4) in families A, B, and C as well as some less frequently

TABLE 2. Serotypes of isolates in family outbreaks

Family	Patient (age [yr]) and animal contact	Serotype of isolate
A	Adult male (65)	1
	Adult female (61)	1
	Chicken	1
B	Male child	2
	Mother	2
	Father	2
C	Mother (32)	4
	Female child (6)	4
D	Female child (6)	5
	Female child (2)	5
E	Male child (9)	11
	Female child (6)	11
F	Mother (29)	31
	Male child (10)	31
G	Child (2)	20
	Child (0.25)	45

TABLE 3. Serotype distribution of *C. jejuni* isolates

Serotype	No. of isolates		Serotype	No. of isolates	
	Separate isolations[a]	Epidemiologically linked[b]		Separate isolations[a]	Epidemiologically linked[b]
1	19 (11.3)	5 + 2[c]	19	2 (1.2)	
1/4	1 (0.6)		20	1 (0.6)	
2	20 (11.9)	2	21	2 (1.2)	
2/4	1 (0.6)		22	1 (0.6)	
2/13	1 (0.6)		23	1 (0.6)	
3	6 (3.6)		23/26	2 (1.2)	
4	21 (12.5)	1	24	1 (0.6)	
4/13/16	1 (0.6)		28	1 (0.6)	
4/13/34	1 (0.6)		31	7 (4.2)	1
4/16	1 (0.6)		33	1 (0.6)	
4/19	1 (0.6)		34	2 (1.2)	
5	9 (5.4)	1	37	1 (0.6)	
5/30	1 (0.6)		41	5 (3.0)	
5/31	2 (1.2)		42	3 (1.8)	
6/7	2 (1.2)		43[d]	4 (2.4)	
8	8 (4.8)		45[d]	6 (3.6)	
10	2 (1.2)		46[d]	1 (0.6)	
11	3 (1.8)	1	47	3 (1.8)	
13/16	8 (4.8)		48	1 (0.6)	
13/19	1 (0.6)		50	3 (1.8)	
14	2 (1.2)		51	1 (0.6)	
16	1 (0.6)		53[d]	1 (0.6)	
18	6 (3.6)				

[a] Separate isolations includes sporadic isolates plus one isolate from each outbreak. Percentages are shown within parentheses.

[b] Includes all but one isolate from each outbreak.

[c] Five isolates were from community outbreak (Table 1), and two were from family B outbreak (Table 2).

[d] New serotypes identified in this study.

occurring types (11 and 31) in families E and F. Isolates from siblings in family G were found to be different in serotype (20 and 45). Repeated resubmissions for serotyping of these two isolates (under coded designations) produced the same results. It should be noted that the isolate of serotype 20 was hippurate negative and the other of serotype 45 was hippurate positive. Thus, differentiation by serotyping was consistent with differentiation by biotyping. The lack of correlation between serobiotyping and the clinically expected results for family G remains unexplained.

Prevalence of serotypes among British Columbia isolates. To show prevalence of each serotype more accurately, only one isolate from each outbreak was considered as contributing to the prevalence of that particular serotype. Hence, only 168 of the 181 serotyped isolates were taken into account in determining the serotype distribution (Table 3). Isolates sent for repeat serotyping were not included. The most common isolates were serotype 4 followed by serotypes 1 and 2. This contrasts with results from a previous study in which it was found that isolates of serotypes 2 and 1 were most common (21). More than one-half of the isolates (50.3%) fell into six serotypes (1, 2, 4, 5, 8, and 13/16). Similar concentrations of isolates within a small number of serotypes were also noted previously in two hospitals (21). Isolates that reacted in both antisera 13 and 16 constituted 5.0% of the total and, as in the previous study, were the most frequently occurring of the multiply agglutinating types. Only 15 others (9.0%) reacted in two or more antisera. The number that reacted only in one antiserum therefore formed the largest group (86%). No untypable isolates were recorded in this study. Twelve isolates that were initially classified as untypable were found to belong to one or another of four new types (serotypes 43, 45, 46, and 53) identified in this study.

DISCUSSION

We report the suitability of a serotyping method for discriminating between strains of *C. jejuni*. The passive hemagglutination technique used was previously reported to separate strains of *C. jejuni* on the basis of thermostable antigens (15, 21). With this method, only the heat-extracted, soluble antigens having affinity for the erythrocyte surface are measured, thereby circumventing the problem of autoagglutinability found

with *Campylobacter* cell suspensions (1, 17). The large size of the erythrocyte, acting as antigen carrier, increases the sensitivity of detecting antigen-antibody reactions compared with direct bacterial agglutination.

The serotyping results of strains from a community outbreak correlated well with clinical data and with biochemical and antibiotic sensitivity test results. This collaborative study was therefore undertaken to further assess the reliability of the serotyping scheme and to extend knowledge of the epidemiology of the species with serotyping results.

In the course of this study, 181 isolates were typed and assigned to 1 or more of 45 serotypes. In six separate instances, family outbreaks were found to be caused by a serotype common to all members of the particular family or to their animal contact. In another family, serotyping separated isolates of different biotypes. Results of these smaller outbreaks confirmed the effectiveness of serotyping for epidemiological purposes.

The serotyping scheme is now capable of identifying 55 different antigenic specificities by means of the same number of antisera. Some isolates were found to react in two or more antisera against serotypes not previously known to be related. Thus, some strains possess complexes of antigens. Isolates with such multiple specificities will be useful in defining new serotypes in a scheme that is expected to be expanded to include cross-absorbed antisera as in the well-known Kauffman-White scheme for *Salmonella* spp.

It is clear from this study and from others that there are considerable geographical differences among the serotypes in their frequency of isolation (15, 21). It can be expected that the number of serotypes will continue to increase as isolates from more widely separated sources are examined. The detection of four new serotypes (43, 45, 46, and 53) among only 181 isolates of this study illustrates the point. Human infections are not restricted to particular serotypes. Sporadic cases and outbreaks may be caused by frequently isolated types such as those of serotypes 1, 2, and 4 or by infrequently occurring types such as 11 and 31.

More work is necessary to clarify the epidemiological significance of *Campylobacter* infections in human and animal populations. This study has shown the applicability of the passive hemagglutination technique to this task. Further evaluations of the scheme are therefore warranted and should be conducted independently by other laboratories who will undertake the tasks of reconstituting the scheme and testing it in practice. Presently, the Division of Laboratories of the Ministry of Health in British Columbia is one of the laboratories that is participating in this testing program.

ACKNOWLEDGMENTS

We thank G. Geddes, T. Matheson, and K. Tanner of the British Columbia Ministry of Health Laboratories and M. M. Goodbody at the University of Toronto for skilled technical assistance.

This research was supported by the British Columbia Health Care Research Foundation and Health and Welfare Canada.

LITERATURE CITED

1. **Abbott, J. D., B. Dale, J. Eldridge, D. M. Jones, and E. M. Sutcliffe.** 1980. Serotyping of *Campylobacter jejuni/coli*. J. Clin. Pathol. **33**:762–766.
2. **Blaser, M., J. Cravens, B. W. Powers, and W. L. Lang.** 1978. Campylobacter enteritis associated with canine infection. Lancet **ii**:979–981.
3. **Blaser, M., F. M. LaForce, N. A. Wilson, and W. L. L. Wang.** 1980. Reservoirs for human campylobacteriosis. J. Infect. Dis. **141**:665–669.
4. **Bokkenheuser, V. D., N. J. Richardson, J. H. Bryner, D. J. Roux, A. B. Schutte, H. J. Koornhof, I. Frieman, and E. Hartman.** 1979. Detection of enteric campylobacteriosis in children. J. Clin. Microbiol. **9**:227–232.
5. **Bruce, D., W. Zochowski, and I. R. Ferguson.** 1977. Campylobacter enteritis. Br. Med. J. **2**:1213.
6. **Butzler, J. P., and M. B. Skirrow.** 1979. Campylobacter enteritis. Clin. Gastroenterol. **8**:737–765.
7. **Cary, S. G., and E. B. Blair.** 1964. New transport medium for shipment of clinical specimens. I. Faecal specimens. J. Bacteriol. **88**:96–98.
8. **Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon.** 1972. Acute enteritis due to related vibrio: first positive stool cultures. J. Infect. Dis. **125**:390–392.
9. **Disease Surveillance, British Columbia Ministry of Health.** 1981. Waterborne campylobacteriosis **2**:1–2.
10. **Grant, I. H., N. R. Richardson, and V. D. Bokkenheuser.** 1980. Broiler chickens as potential source of *Campylobacter* infections in humans. J. Clin. Microbiol. **11**:508–510.
11. **Haley, C. E., R. A. Gunn, J. M. Hughes, E. C. Lippy, and G. F. Craun.** 1980. Outbreaks of waterborne disease in the United States, 1978. J. Infect. Dis. **141**:794–797.
12. **Kaplan, R. L.** 1980. Campylobacter, p. 235–241. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
13. **Karmali, M. A., and P. C. Fleming.** 1979. *Campylobacter enteritis* in children. J. Pediatr. **94**:527–533.
14. **Kosunen, T. U., D. Danielsson, and J. Kjellander.** 1980. Serology of *Campylobacter fetus* ss. *jejuni* ("related" campylobacters). Acta Pathol. Microbiol. Scand. Sect. B **88**:207–218.
15. **Lauwers, S., L. Vlaes, and J. P. Butzler.** 1981. Campylobacter serotyping and epidemiology. Lancet **i**:158–159.
16. **Leapers, S., and R. J. Owen.** 1981. Identification of catalase-producing *Campylobacter* species based on biochemical characteristics and on cellular fatty acid compositions. Curr. Microbiol. **6**:31–35.
17. **Lior, H., D. L. Woodward, J. A. Edgar, and L. J. LaRoche.** 1981. Serotyping by slide agglutination of *Campylobacter jejuni* and epidemiology. Lancet **ii**:1103–1104.
18. **Mentzing, L.-O.** 1981. Waterborne outbreaks of campylobacter enteritis in central Sweden. Lancet **ii**:352–354.
19. **Park, C. E., Z. K. Stankiewicz, J. Lovett, and J. Hunt.** 1981. Incidence of *Campylobacter jejuni* in fresh eviscerated whole market chickens. Can. J. Microbiol. **27**:841–842.

20. **Pearson, A. D., W. G. Suckling, I. D. Ricciardi, M. Knill, and E. Ware.** 1977. Campylobacter-associated diarrhoea in Southampton. Br. Med. J. **2**:955–956.
21. **Penner, J. L., and J. N. Hennessy.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12**:732–737.
22. **Robinson, D. A., W. J. Edgar, G. L. Gibson, A. A. Matchett, and L. Robertson.** 1979. Campylobacter enteritis associated with consumption of unpasteurized milk. Br. Med. J. **1**:1171–1173.
23. **Skirrow, M. B.** 1977. Campylobacter enteritis: a "new" disease. Br. Med. J. **2**:9–11.
24. **Svedhem, A., and G. Norkrans.** 1980. *Campylobacter jejuni* enteritis transmitted from cat to man. Lancet **i**:713–714.
25. **Yanagisawa, S.** 1980. Large outbreak of campylobacter enteritis among school children. Lancet **ii**:153.