JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1986, p. 592–594
0095-1137/86/030592-03$02.00/0

Vol. 23, No. 3

Distribution and Serotypes of *Campylobacter jejuni* and *Campylobacter coli* in Enteric *Campylobacter* Strains Isolated from Children in the Central African Republic

M. C. GEORGES-COURBOT,* C. BAYA, A. M. BERAUD, D. M. Y. MEUNIER, AND A. J. GEORGES

Institut Pasteur de Bangui, Bangui, Central African Republic

Received 26 August 1985/Accepted 9 December 1985

One hundred eighty-five enteric *Campylobacter* strains isolated from diarrheic or healthy children in Bangui (Central African Republic) were studied to determine their species and serotypes. *C. coli* was identified in 38.9% of all strains and in 43.9% of strains from diarrheic children. By the hemagglutination technique for heat-stable antigens, 73.5% of the strains could be serotyped. Of the typeable strains, 75% were distributed among 13 more frequent serotypes. *C. coli* serotype Pen 37,56 was the most common serotype from diarrheic children.

Since practical techniques for the isolation of *Campylobacter jejuni* and *C. coli* have become available, numerous studies on the etiological role of these bacteria in human diarrhea in many countries have been reported. The results show a different epidemiological pattern in developed countries than in developing countries. In developed countries, the incidence of *Campylobacter* infection is low, and there are few healthy carriers (3, 23). In developing countries, the incidence of *Campylobacter* infection in diarrhea is higher, and there are numerous healthy carriers (2, 4, 7). The prevalence of *Campylobacter* infection in Bangui (Central African Republic) is similar to that in other developing countries. An epidemiological study conducted in Bangui from March 1981 to February 1982 showed *C. jejuni* and *C. coli* to be the third most prevalent enteric pathogens identified in the stools of diarrheic children. The study also demonstrated that many instances of asymptomatic carriers occur, such that the difference between the two groups is not significant (6). The serotype of an isolate can be used as a marker to study the epidemiology of *Campylobacter* infections. Several techniques have been recently developed for serotyping *C. jejuni* and *C. coli*: a passive hemagglutination technique based on heat-stable antigens (13, 20), a slide agglutination technique based on heat-labile antigens (15), and a direct immunofluorescence technique (9).

In the present paper, we present the results of a study of enteric *Campylobacter* strains isolated from the stools of children in Bangui from May 1982 to October 1984. For all isolates, we determined the species and serotype by the passive hemagglutination technique of Penner and Hennessy (20) to determine if there were differences in strains between diarrheic and healthy children.

MATERIALS AND METHODS

Isolation and identification. All *Campylobacter* strains were isolated from fecal specimens of diarrheic or healthy children under 15 years of age, and all strains were from sporadic cases. We did not observe an outbreak during that period. Of the diarrheic children, 81% were recruited in the city of Bangui (30% in the pediatric department of the hospital and 51% in a medical day care center) and 19% in a rural village. The healthy children came from the same care center or belonged to a cohort of children followed by our laboratory from birth to 2 years of age.

Each fecal specimen was plated on Butzler medium (Oxoid Ltd.) (5). The plates were incubated at 42°C in an anaerobic jar with a gas-generating pack but without catalyst. After incubation for 48 h, enteric campylobacters were identified by colony morphology, Gram stain, motility by dark-field illumination, and the presence of catalase and oxidase (16).

Each strain was tested for susceptibility to nalidixic acid with 30-μg disks; this method distinguishes *C. laridis* (nalidixic acid resistant) from *C. jejuni* and *C. coli* (nalidixic acid susceptible) (24). Any zone of inhibition of growth was considered susceptible. The distinction between *C. jejuni* and *C. coli* was made on the basis of the rapid hippurate hydrolysis technique described by Harvey (8). Hippurate-positive strains were classified as *C. jejuni* and hippurate-negative strains as *C. coli* (24).

Serotyping. Immune antisera were prepared in our laboratory against Penner reference strains 1 to 56 by immunizing rabbits by the technique described by Penner and Hennessy (20) modified by using formalinized bacteria in place of live bacteria for the first three immunizations and live organisms for the fourth and fifth injections. Penner reference strains were provided by G. Morris from the *Campylobacter* reference laboratory at the Centers for Disease Control, Atlanta, Ga. Each antiserum was tested against all 56 reference strains. The homologous titers of the immune sera varied from 1/160 to 1/10,240. Nonabsorbed sera were used to serotype strains. The serotype of each strain of *C. jejuni* and *C. coli* was determined by testing each strain in the 56 immune antisera diluted 1/40 in microtitration plates as described by Penner and Hennessy (20). For each positive reaction, the titer was determined by serial twofold dilutions of the antisera in microtitration plates. Dilutions were carried out with manual microdilutors. Weak and strong antigen activities were determined by comparing the titer of the tested strain with the titer of the reference (immunizing) strain (20). Strains were retested if only a weak reaction or no reaction occurred, and strains consistently showing only weakly reacting antigens were described as weak. Strains were not retested if strong antigens were present; therefore, transient antigens (19) were not detected. The serotype was

* Corresponding author.

TABLE 1. Age distribution of diarrheic and healthy *Campylobacter*-infected children

Age	No. of diarrheic cases (%)	No. of healthy cases (%)
0–11 mo	49 (39.8)	47 (75.8)
12–23 mo	52 (42.3)	10 (16.1)
2–15 yr	22 (17.9)	5 (8.1)

indicated by listing all reactive antisera in order by strength of titer, with the antiserum giving the strongest reaction listed first.

RESULTS

We studied 185 enteric *Campylobacter* strains, 123 from diarrheic children and 62 from healthy children. The age distribution of the two groups of children is summarized in Table 1. For 64% of the diarrheic children from whose feces enteric campylobacters were isolated, they were the only enteropathogens.

None of the strains were resistant to nalidixic acid; therefore, none were identified as *C. laridis*. All strains were *C. jejuni* or *C. coli*. The distribution of the two species is summarized in Table 2, with 61.1% of the strains identified as *C. jejuni* and 38.9% as *C. coli*.

Serotyping results for each strain are listed in Table 3. The immune antisera we prepared serotyped 136 (73.5%) strains; 26% of the strains of symptomatic carriers and 27.4% of the strains of controls were untypeable. Thirty-five different serotypes were recognized; however, 14 serotypes were identified only once. Of the typeable strains, 102 (75%) were distributed among 13 serotypes (Table 3). We included in the typeable strains 12 which reacted weakly in antisera 8 (8 strains) and 41 (4 strains). These reactions may represent cross-reactions with an unknown serotype not yet identified. Fifty-two (38.2%) of the strains agglutinated in two or more antisera. Thirty-one strains distributed in six different serotypes reacted in both *C. jejuni* and *C. coli* antisera (Table 3; serotypes Pen 5,31,39; 24,19; 34,26,40; 34,40,53; 37,56; and 49,53).

Only one strain, *C. coli* serotype Pen 37,56 occurred much more frequently among diarrheic children (18 cases) than in the control group (2 cases), the difference being statistically significant ($P < 0.02$; Fisher exact test). Three other strains, *C. jejuni* serotypes Pen 5, 8, and 53, were found more frequently in diarrheic than in healthy children, but the difference was not statistically significant.

DISCUSSION

We observed a high percentage of *C. coli* in the enteric *Campylobacter* strains isolated in Bangui. The occurrence of *C. coli* was 38.9% of all strains identified compared with 43.9% of strains of symptomatic subjects only. *C. coli* is commonly isolated from pigs (25); however, isolation from humans is infrequent and has been reported to be 3.2% by Karmali et al. (12), 5% by Skirrow and Benjamin (24), 13.4% by Kapperud et al. (11), and 17.6% by Lior (14). This is the first report of the distribution of the two species *C. jejuni* and *C. coli* in Africa, and the frequency of occurrence of the two species seems to be different from that found elsewhere.

We were able to serotype 136 strains isolated in the Central African Republic (73.5% of the strains) with immune sera prepared against strains isolated elsewhere; 26.5% of the strains were untypeable and may represent new serotypes. We found a high diversity of serotypes of *C. jejuni* and *C. coli* in Bangui; 35 different serotypes were identified. However, 75% of the strains belonged to 13 serotypes. Among these most frequent serotypes, six serotypes, Pen 2, 3, 5, 8, 13, and 23,36 have been previously identified by other authors as occurring frequently (12, 18, 21). They represent 44 strains, that is, 23.8% of all the tested strains. We did not find strains of serotype Pen 1 or 4, which are reported by those authors as frequent. On the other

TABLE 2. Distribution of *C. jejuni* and *C. coli* among diarrheic and healthy children

Species	No. (%) among diarrheic cases	No. (%) among healthy cases	No. (%) among all cases
C. jejuni	69 (56.1)	44 (71.0)	113 (61.1)
C. coli	54 (43.9)	18 (29.0)	72 (38.9)

TABLE 3. Serotypes of *C. jejuni* and *C. coli* isolated in Bangui[a]

Serotype[b]	No. of isolates of *C. jejuni* from:		No. of isolates of *C. coli* from:	
	Patients	Normals	Patients	Normals
1,3[c]	4	3		1
2[c]	2	4	1	
3[c]	3	2	2	
3,17	1			
5[c]	6	2	1	
5,2				1
5,3	1			
5,31	1			
5,31,39			1	
8w[c,d]	7	1		
13[c,e]	2	4		
17	1	2		
19	1		1	
24			2	
24,19			1	1
29	1			
30			1	2
31			1	
34[f]		1	1	1
34,26,40				1
34,40,53			1	
35	1			
36,23[c]	2	2	3	
37,56[c]	1		18	2
40		1		
40,53	1			
41w[c,d]		4		
43	1			
46			2	1
46,49			1	
48	1		1	
49[c]			3	3
49,53[c]	1		3	1
51[c]			2	2
53[c]	7	2		1

[a] Of the *C. jejuni* isolates from patients, 45 were typeable and 24 were not; of those from healthy children, 28 were typeable and 16 were not. Of the *C. coli* isolates from patients, 46 were typeable and 8 were not; of those from healthy children, 17 were typeable and 1 was not.

[b] *C. coli* antisera are underlined.

[c] Most frequently identified serotypes.

[d] Weak reaction only.

[e] Strain may react in one or more of antisera 4, 13, 16, and 50.

[f] Strain may react in antisera 34 and 26.

hand, *C. coli* serotype Pen 37,56, which is infrequently isolated elsewhere, was the enteric campylobacter most frequently isolated from the stools of diarrheic children in Bangui and represented 10.8% of all the strains. The different isolates of these strains did not seem to be related, since they were isolated at different times during the 2 years from 14 different areas of the city and also from a rural area. Half of the families in which these strains were identified had an individual water supply (well). This strain appears to be interesting for two reasons. (i) It was isolated with a significantly higher frequency in the patient group than in the control group. (ii) The species *C. coli* is rarely isolated from human specimens. Of the serotypeable strains, 22.8% were found to react in both *C. jejuni* and *C. coli* antisera. This observation has been reported previously, although not as frequently as in the Bangui study (10–12).

The higher number of healthy carriers of *C. jejuni* and *C. coli* in developing countries remains unexplained. Two possibilities can be considered to explain this difference: lower pathogenicity of the strains from developing countries and early immunization of children due to the low level of hygiene in these countries, thereby allowing a high prevalence of the bacterium. Two different mechanisms are now thought to explain *Campylobacter* pathogenicity: enteroinvasive ability and elaboration of toxins (3, 5, 22). A study conducted in India, where the number of healthy carriers of enteric campylobacter is very high, has shown no difference in enterotoxigenicity between the strains isolated from diarrheic children and those from healthy children (17). A recent study conducted by Blaser et al. (1) makes the second possibility more probable. They have shown that the level of enteric *Campylobacter* serum-specific antibodies in healthy children was higher in a developing country (Bangladesh) than in a developed country (the United States). In our study, we identified a predominant serotype of *C. coli* in patients. Further studies, such as pathogenicity tests and an understanding of the immune status of the children, are needed to clarify the role of *C. jejuni* and *C. coli* in diarrhea in developing countries.

ACKNOWLEDGMENTS

We thank George K. Morris and Charlotte M. Patton for their consultation and advice during the study and for having reviewed the manuscript.

LITERATURE CITED

1. **Blaser, M. J., R. E. Black, D. J. Duncan, and J. Amer.** 1985. *Campylobacter jejuni*-specific antibodies are elevated in healthy Bangladeshi children. J. Clin. Microbiol. **21**:164–167.
2. **Blaser, M. J., R. I. Glass, M. I. Huq, B. Stoll, G. M. Kibriya, and A. R. M. A. Alim.** 1980. Isolation of *Campylobacter fetus* subsp. *jejuni* from Bangladeshi children. J. Clin. Microbiol. **12**:744–747.
3. **Blaser, M. J., and L. B. Reller.** 1981. Campylobacter enteritis. N. Engl. J. Med. **305**:1444–1452.
4. **Bokkenheuser, V. D., N. J. Richardson, J. H. Bryner, D. J. Roux, A. B. Schutte, H. J. Koornhof, I. Freiman, and E. Hartman.** 1979. Detection of enteric campylobacteriosis in children. J. Clin. Microbiol. **9**:227–232.
5. **Butzler, J. P., and M. B. Skirrow.** 1979. Campylobacter enteritis. Clin. Gastroenterol. **8**:737–765.
6. **Georges, M. C., I. K. Wachsmuth, D. M. Y. Meunier, N. Nebout, F. Didier, M. R. Siopathis, and A. J. Georges.** 1984. Parasitic, bacterial, and viral enteric pathogens associated with diarrhea in the Central African Republic. J. Clin. Microbiol. **19**:571–575.
7. **Glass, R. I., B. J. Stoll, M. I. Huq, M. J. Struelens, M. Blaser, and A. K. M. G. Kibriya.** 1983. Epidemiologic and clinical features of endemic *Campylobacter jejuni* infection in Bangladesh. J. Infect. Dis. **148**:292–296.
8. **Harvey, S. M.** 1980. Hippurate hydrolysis by *Campylobacter fetus*. J. Clin. Microbiol. **11**:435–437.
9. **Hebert, G. A., D. G. Hollis, R. E. Weaver, A. G. Steigerwalt, R. M. McKinney, and D. J. Brenner.** 1983. Serogroups of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter fetus* defined by direct immunofluorescence. J. Clin. Microbiol. **17**:529–538.
10. **Itoh, T., K. Saito, Y. Yanagawa, S. Sakai, and M. Ohashi.** 1982. Serological typing of thermophilic campylobacter isolated in Tokyo, p. 106–110. *In* D. G. Newell (ed.), *Campylobacter*. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster, England.
11. **Kapperud, G., J. Lassen, S. Lauwers, and O. Rosef.** 1984. Serotyping and biotyping of *Campylobacter jejuni* and *Campylobacter coli* from sporadic cases and outbreaks in Norway. J. Clin. Microbiol. **19**:157–160.
12. **Karmali, M. A., J. L. Penner, P. C. Fleming, A. Williams, and J. N. Hennessy.** 1983. The serotype and biotype distribution of clinical isolates of *Campylobacter jejuni* and *Campylobacter coli* over a three-year period. J. Infect. Dis. **147**:243–246.
13. **Lauwers, S., L. Vlaes, and J. P. Butzler.** 1981. Campylobacter serotyping and epidemiology. Lancet **i**:158.
14. **Lior, H.** 1984. New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and "*Campylobacter laridis*." J. Clin. Microbiol. **20**:636–640.
15. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J. Clin. Microbiol. **15**:761–768.
16. **Luechtefeld, N. W., W. L. L. Wang, M. J. Blaser, and L. B. Reller.** 1981. *Campylobacter fetus* subsp. *jejuni*: background and laboratory diagnosis. Lab. Med. **12**:481–487.
17. **Mathan, V. I., D. P. Rajan, F. A. Klipstein, and R. F. Engert.** 1984. Enterotoxigenic *Campylobacter jejuni* among children in south India. Lancet **ii**:981.
18. **McMyne, P. M. S., J. L. Penner, R. G. Mathias, W. A. Black, and J. N. Hennessy.** 1982. Serotyping of *Campylobacter jejuni* isolated from sporadic cases and outbreaks in British Columbia. J. Clin. Microbiol. **16**:281–285.
19. **Patton, C. M., T. J. Barrett, and G. K. Morris.** 1985. Comparison of Penner and Lior methods for serotyping *Campylobacter* spp. J. Clin. Microbiol. **22**:558–565.
20. **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.
21. **Penner, J. L., J. N. Hennessy, and R. V. Congi.** 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of the thermostable antigens. Eur. J. Clin. Microbiol. **2**:378–383.
22. **Ruiz-Palacios, G. M., N. I. Torres, B. R. Ruiz-Palacios, J. Torres, E. Escamilla, and J. Tamayo.** 1983. Cholera-like enterotoxin produced by *Campylobacter jejuni*. Characterization and clinical significance. Lancet **ii**:250–254.
23. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
24. **Skirrow, M. B., and J. Benjamin.** 1980. Differentiation of enteropathogenic *Campylobacter*. J. Clin. Pathol. **33**:1122.
25. **Skirrow, M. B., and J. Benjamin.** 1982. The classification of "thermophilic" campylobacters and their distribution in man and domestic animals, p. 40–44. *In* D. G. Newell (ed.), *Campylobacter*: epidemiology, pathogenesis, and biochemistry. MTP Press Ltd., Lancaster, England.