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Serotype Distribution of *Campylobacter jejuni* and *Campylobacter coli* Isolated from Hospitalized Patients with Diarrhea in Central Australia

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***Campylobacter jejuni* and/or *Campylobacter coli* was cultured from 218 of 1,078 patients of all age groups admitted to Alice Springs Hospital, Alice Springs, central Australia, between July 1988 and June 1989 for treatment of diarrhea. One hundred sixty-six *Campylobacter* colonies from 127 patients were subjected to O serotyping by using the Penner typing scheme. All except 29 colonies could be serotyped. A total of 46 serotypes were identified, and the predominant serotypes were O:8,17, O:22, O:1,44, and O:19. A large proportion of colonies reacted with more than one antiserum, and nine serotypes had antigenic compositions not observed previously. Several patients had multiple infections with more than one serotype, and some patients were shown for the first time to be infected with up to three different serotypes. Repeated reinfections with different serotypes were seen in some patients. In some patients, provided it was not due to reinfection with the same serotype, long-term excretion of the same serotype was seen, and for the first time, one patient showed evidence of excretion of the same serotype for up to 73 days.**

Campylobacter organisms occur worldwide in humans, animals, and the environment (11, 13, 17). Among the various species of the genus *Campylobacter*, *Campylobacter jejuni* and *Campylobacter coli* have been firmly established as important diarrheal pathogens in both developing and developed countries (1a, 2).

The burden of diarrhea in the aboriginal population of central Australia, who live in isolated settlements cut off from the mainstream population, is very heavy. A study to determine the prevalence of serotypes, prevalence of mixed infections with more than one serotype, duration of excretion of serotypes, and acquisition of new serotypes of *C. jejuni* and *C. coli* in hospitalized patients (of predominantly aboriginal people) with diarrhea in central Australia was initiated as a means of understanding the dynamics of infections due to these organisms.

MATERIALS AND METHODS

Population. The aboriginal people of central Australia live in approximately 70 settlements spread over a wide area. The distance between settlements varies from 20 to 700 km. Contact among communities is infrequent, as roads linking them are almost nonexistent. The populations of settlements vary from fewer than 100 to more than 1,000 people per settlement. Since these people live in a transitional society, their hygienic practices are primitive by Western standards. A 170-bed hospital at Alice Springs, central Australia, is the only hospital providing in-patient care to these people. The study was conducted with hospitalized patients of all age groups who had diarrhea and were admitted between July 1988 and June 1989.

Samples. Stool specimens were obtained from 1,078 patients. Eighty-one percent of patients belonged to the aboriginal race, and the remaining patients belonged to other races. Several patients had multiple admissions corresponding to different episodes of diarrhea. For isolation of *Campylobacter* spp., the stool was streaked on *Campylobacter* blood-free selective agar with cefoperazone selective supplement (Oxoid), and the plates were incubated at 42°C in an anaerobic jar with catalyst (BBL) in an increased CO_2 atmosphere generated by *Campylobacter* gas-generating sachet (Oxoid). The plate was examined after 48 h, and if negative for *Campylobacter* spp., it was discarded. A filter technique was simultaneously used for the isolation of *Campylobacter* spp. (15). Briefly, a 1:10 dilution of approximately 50 μl of fecal suspension in sterile normal saline was inoculated onto a 0.65-μm-pore-size membrane filter (Sartorius) placed on a 5% sheep blood agar plate. After 30 min, the membrane was removed, and the plate was incubated at 37°C in an increased-CO_2 atmosphere generated as described above. The plate was examined for *Campylobacter* spp. after 48 h and every day thereafter until day 5. From both media, different colony morphotypes were picked for identification.

Bacteria. *Campylobacter* isolates were identified by standard procedures, such as morphology after Gram stain and characteristic motility, catalase, and oxidase tests (13). *C. jejuni* and *C. coli* were further characterized by susceptibility to cephalothin and nalidixic acid and by the hippurate hydrolysis test (4, 14). All the *Campylobacter* isolates were stocked at −70°C in brain heart infusion broth (Oxoid) with 15% glycerol.

Application of the filter technique resulted in the isolation of *C. jejuni* subsp. *doylei*, *Campylobacter fetus*, "*Campylobacter upsaliensis*," *Campylobacter hyointestinalis*, *Campylobacter laridis*, and uncharacterized *Campylobacter* spp. The characterization of these isolates will be reported in a separate communication (1).

O serotyping. After isolates were identified to species level, antigenic extracts were prepared according to procedures described previously (8) and shipped to the Department of Microbiology, University of Toronto, where they

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TABLE 1. Serotype distribution of *C. jejuni* and *C. coli* from hospitalized diarrheal patients in central Australia, July 1988 through June 1989

Species and serotype	No. of:	
	Patients harboring serotype (n = 127)	Settlements sharing serotype
C. jejuni		
1,44	10	6
3	1	1
3_w	3	2
4	1	1
4,64[a]	1	1
4,13,16,22,65[a]	1	1
5,13[a]	1	1
5,31	3	2
8_w	1	1
8,17	17	8
8_w,17_w	1	1
8,17,19[a]	1	1
10_w	1	1
$12,44_w$	1	1
13,50	1	1
13,63[a]	1	1
17_w	2	1
18	3	2
19	8	7
19_w	2	2
19,29[a]	1	1
19,38[a]	1	1
21	1	1
22	12	7
22,64[a]	3	3
23	2	2
23,36	1	1
23,53	1	1
29,42	2	1
31	1	1
37	4	2
37,56	1	1
41	3	3
44	4	3
44_w	2	2
45	1	1
46	1	1
46_w	1	1
49	1	1
49_w	2	2
50,63[a]	1	1
58	1	1
63	1	1
66	4	2
$MCH66_w$[b]	1	1
C. coli 54	2	2

[a] New serotype.
[b] Not yet assigned a numerical designation.

were used to sensitize sheep erythrocytes for O serotyping by the passive hemagglutination technique. The number of antisera for serotyping was increased from the list reported earlier (10) by including antisera against recently defined O serotypes O:60 to O:66 and an antiserum against strain MCH66, which has not yet been confirmed to have a unique serotype. The O serotype assigned to an isolate was based on the antisera in which passive hemagglutination titers greater than 1:40 were observed. Titers of 1:40 and 1:80 were considered weak (w) reactions.

TABLE 2. Mixed infections with *C. jejuni* and *C. coli* and with different serotypes of *C. jejuni* in hospitalized diarrheal patients in central Australia, July 1988 through June 1989[a]

Patient no.	Species	Serotype
457	*C. jejuni*	22
	C. jejuni	22,64
565	*C. jejuni*	58
	C. jejuni	8,17
657	*C. jejuni*	19
	C. jejuni	19,38
672	*C. jejuni*	NT[b]
	C. jejuni	1,44
689	*C. jejuni*	44
	C. jejuni	1,44
695	*C. jejuni*	45
	C. jejuni	1,44
724	*C. jejuni*	NT
	C. jejuni	19
835	*C. jejuni*	NT
	C. jejuni	8,17
573	*C. coli*	54
	C. jejuni	NT
	C. jejuni	22,64
617	*C. jejuni*	NT
	C. jejuni	3_w
	C. jejuni	66
648	*C. jejuni*	1,44
	C. jejuni	8,17
	C. jejuni	22,64
654	*C. coli*	54
	C. jejuni	3_w
	C. jejuni	66
760	*C. jejuni*	NT
	C. jejuni	5,31
	C. jejuni	41

[a] Not included are six patients who had mixed infections with *C. jejuni* and *C. coli* but whose isolates were not serotyped.
[b] NT, not typeable.

RESULTS

Of the 218 individuals harboring *Campylobacter* spp., 179 were infected with *C. jejuni*, 31 were infected with *C. coli*, and 8 were infected with both *C. jejuni* and *C. coli*. Serotyping of 166 colonies (162 *C. jejuni* and 4 *C. coli*) from 127 patients was attempted. (The isolates from the remaining patients were lost during storage.) The 127 patients were sampled as follows: 114 were sampled once, 9 were sampled twice, 2 were sampled three times, 1 was sampled four times, and 1 was sampled six times. These patients came from 34 different settlements in central Australia. A total of 137 colonies (77%) could be assigned to a serotype. The serotype distribution in patients and settlements is shown in Table 1. The most common *C. jejuni* serotypes were O:8,17, O:22, O:1,44, and O:19, and a large proportion of patients had strains which reacted with more than one antiserum. Several serotypes were shared by people living in different settlements.

In addition to the 8 patients who had mixed infections with *C. jejuni* and *C. coli*, serotyping revealed mixed infections with two or three different serotypes of *C. jejuni* in another 11 patients (Table 2). Table 2 also contains details of infection in two (patients 573 and 654) of the eight subjects who had mixed infections with *C. jejuni* and *C. coli*. The results for the remaining six patients are not included in the table, as serotyping of the isolates from these patients was not attempted.

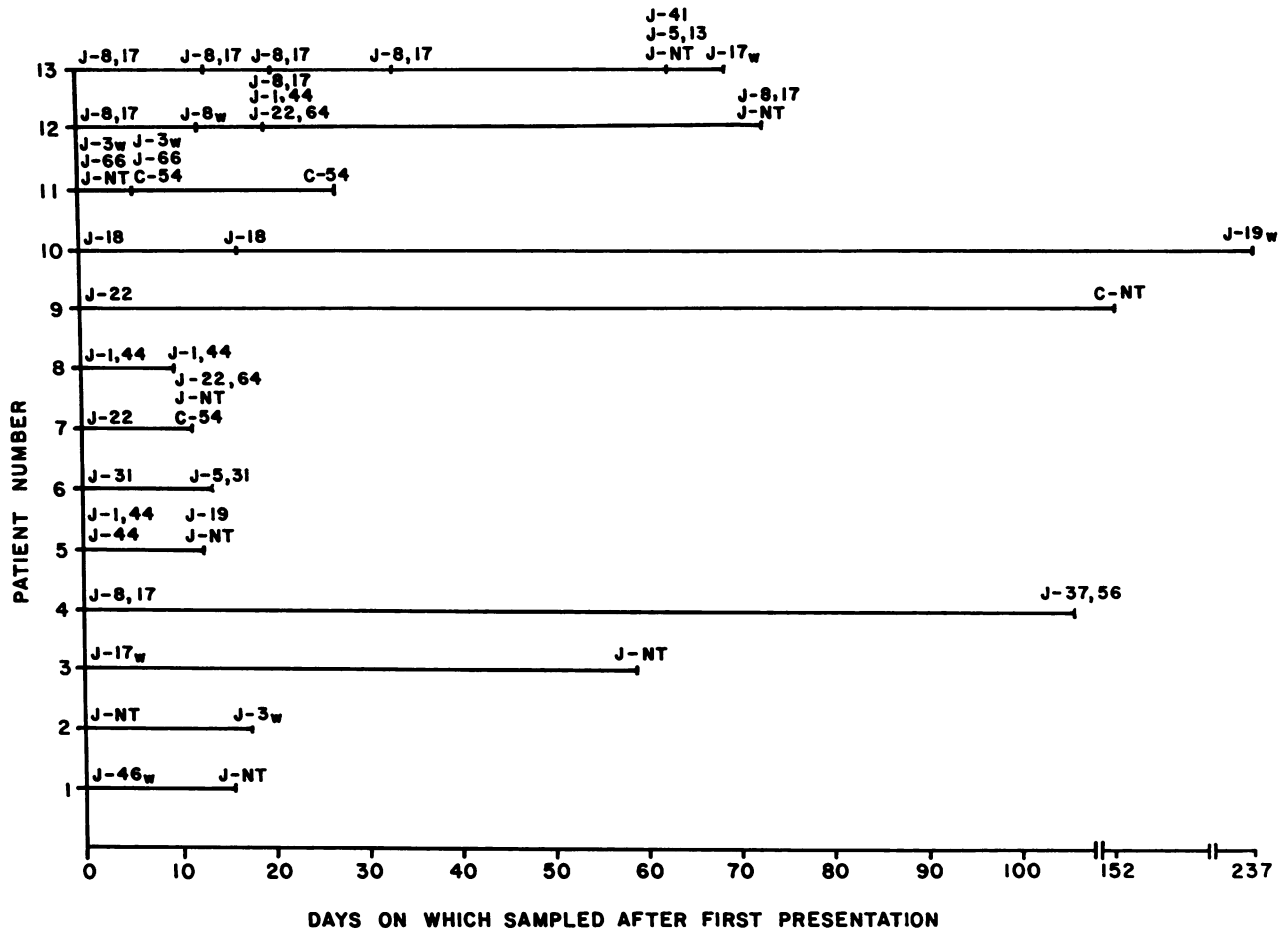

FIG. 1. Duration of excretion of *C. jejuni* and *C. coli* and repeated infections due to these organisms in hospitalized diarrheal patients in central Australia, July 1988 through June 1989. Lengths of the lines represent the time between samplings, and points on the lines represent the days on which samplings were done. The serotypes identified on sampling days are also given. Abbreviation: J, *C. jejuni*; C, *C. coli*; NT, not typeable, w, weak reaction with antiserum.

The serotypes of isolates from 13 patients who had repeated stool cultures are shown in Fig. 1. In nine patients (patients 1 through 9) sampled twice, the interval between two samplings varied from 10 to 152 days. In one of the patients (no. 8) resampled after 10 days, the same serotype was isolated. In the remaining patients, a different serotype(s) from the first was isolated. In one patient (no. 10) sampled three times, the serotypes in the first two samples were the same, but a different serotype was isolated from the third sample. In another patient (no. 11) sampled three times, three *C. jejuni* serotypes were initially present. Six days later, one was replaced by *C. coli* serotype 54, and at the third sampling, only serotype 54 was left. In the patient (no. 12) sampled four times, the same serotype (O:18,17) persisted for 73 days, although additional serotypes appeared and disappeared in between. In the one patient (no. 13) sampled six times, the initial infecting serotype (O:8,17) persisted for 34 days through four samplings. At the fifth sampling, three new serotypes appeared, which were all replaced by yet another serotype ($O:17_w$) 6 days later.

DISCUSSION

Serotyping of the isolates showed very interesting patterns (Table 1). Approximately 18% of the isolates could not be serotyped by the available antisera. This is similar to the findings in developing countries from which isolates have been subjected to O serotyping (3, 6). The antisera against new serotypes O:63 and O:64, which were produced against strains CT 28 and CT 282 obtained from South Africa, identified the new serotypes, and antiserum O:66 against strain CT 35 identified four isolates of this serotype that had been found previously only in patients from South Africa (6). Of the 45 serotypes found among the 113 isolates of *C. jejuni*, 9 had antigenic compositions not observed previously. Curiously, serotype O:2, the most frequently isolated in other studies, was completely lacking in this study, and serotypes O:8,17 and O:22, which have been identified with a frequency of only 0.9 and 0.2% in previous studies of serotype distribution (10), constituted approximately 16 and 12%, respectively, of the isolates in this study. It is clear from this study that distribution of the predominant serotypes and other serotypes varies from region to region (3, 6, 9).

Although shipping of extracts instead of live bacteria for O serotyping has been previously recommended (8), this study is the first, to our knowledge, in which the practice was followed. A major advantage is that work associated with subculturing and purification on arrival of the shipment is not necessary. The extracts may be serotyped immediately or

scheduled for a more convenient time. Furthermore, extracts, in contrast to live cells, do not constitute a hazard to shipping personnel. The method is recommended when the reference laboratory is a great distance from the location of the study and when shipment across international boundaries is required.

In spite of the relative isolation of central Australian settlements from one another, many serotypes were shared by several settlements. Although some serotypes were unique to certain settlements, the significance of this finding could not be assessed, as very few patients from some settlements were studied. Perhaps studies of more patients from these settlements would have revealed sharing of these serotypes.

The extent of simultaneous infection with more than one serotype of *C. jejuni* or *C. coli* is not known apart from one report from South Africa, where some patients were found to be infected with two serotypes (7). In our study, there was evidence of mixed infection in 19 patients. In three of these patients (no. 457, 657, and 689 in Table 2), the two isolates recovered in each case were closely related in serotype, the second isolate differing from the first by an additional determinant. It is conceivable that the acquisition of the second determinant was a result of host-induced modification of the first isolate. Further examination of the isolates by restriction endonuclease analysis to determine whether these cases reflected such antigenic variation was not performed. However, it is clear that isolates from the other patients provided evidence for the occurrence of mixed infection and that five patients were infected with up to three quite distinctly different serotypes. This suggests that *Campylobacter* infection is hyperendemic in this population and that the level of exposure is indeed very high. We suspect that dogs are the source of infection in aboriginal communities, even though no attempts were made to isolate *Campylobacter* spp. from them. It is the cultural practice of aboriginal people to live in close association with numerous dogs, which are mostly malnourished and diseased.

Before serotyping schemes were developed for *Campylobacter* spp., studies in the West suggested that convalescent-phase excretion of *Campylobacter* spp. lasts for 2 to 3 weeks (5, 16). However, a study in South Africa showed long-term infection with *Campylobacter* spp. for up to 9 months to 1 year in some schoolchildren (12). It was very difficult to conclude at that time whether this represented prolonged excretion of the same serotype or repeated infections with different serotypes. The advent of serotyping has shed more light on this topic. In a recent study in Thailand, it was found that the duration of convalescent excretion was 14 days in children more than 1 year old and 8 days in children less than 1 year old. Moreover, it was also found that about one-third of the children are reinfected with a new serotype each week (18). In our study, we found evidence of the same serotype being excreted for as long as 73 days, and at the same time, new serotypes were being acquired in less than 1 week. This suggests that both chronic excretion and repeated reinfections occur with *Campylobacter* spp. in this population. However, it is possible that in some instances, repeated reinfections with the same serotypes could be misinterpreted as prolonged excretion of serotypes. Failure to adequately sample colonies initially would result in missing some serotypes, and if these were picked up at subsequent culture during later admissions, it would appear that infection with a second serotype had occurred. Whatever the case, *Campylobacter* infections are hyperendemic in this population.

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