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Long-Term Infections with *Campylobacter fetus* subsp. *jejuni*

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Seventy-three apparently healthy, rural South African schoolchildren 6 to 8 or 13 to 16 years of age were examined five times over a 16-month period for fecal pathogens. Nine were positive for *Campylobacter fetus* subsp. *jejuni*. The organism was isolated intermittently from six children for at least 9 months and from three children for more than 1 year. Five of the long-term infections occurred among the 46 children aged 6 to 8 years (10.9%) versus one long-term infection among the 27 children aged 13 to 16 years (3.7%). It is not possible with present microbiological techniques to make a clear-cut distinction between reinfected subjects and chronic carriers.

The purpose of the present study was to examine the prevalence of enteric pathogens in schoolchildren living in a district of gradually improving hygienic standards. Before 1958, the drinking water in Tlaseng, a rural village 165 km west of Johannesburg, was obtained from open, shallow surface wells shared by humans and animals (4). Some 5 years later, deep wells were constructed, and in recent years the people were encouraged to use pit privies, rather than open fields, for defecation (9).

Information on the prevalence of salmonellae, shigellae, and intestinal parasites is mainly of local interest and will be reported elsewhere. Included in this study was a search for *Campylobacter fetus* subsp. *jejuni*, which in recent years has been recognized as one of the important causes of gastroenteritis (5, 6, 10). In a recent survey near Johannesburg, the infection was found to be very common but comparatively mild (5). *C. fetus* subsp. *jejuni* was recovered as the sole bacterial pathogen from 31% of 0- to 9-month-old children with diarrhea and from 5% of asymptomatic controls ($P < 0.05$). Curiously, 34 to 40% of children aged 9 to 24 months were positive for *C. fetus* subsp. *jejuni* whether they suffered from acute gastroenteritis or were asymptomatic (5). Although transient infections cannot be ruled out it seems most reasonable to assume that the *C. fetus* subsp. *jejuni* were left over from an earlier acute episode which resulted in clinical, but not bacteriological, cure. The purpose of this investigation is to study the occurrence and duration of *C. fetus* subsp. *jejuni* excretion among apparently healthy children from an environment with a high prevalence of *C. fetus* subsp. *jejuni* diarrhea (5).

MATERIALS AND METHODS

Donors. A total of 120 apparently healthy children aged 6 to 8 or 13 to 16 years were chosen for this study. They were pupils of the Tlaseng School, a rural district approximately 165 km west of Johannesburg. Their parents belonged to the lower socioeconomic group. In view of our previous studies on campylobacteriosis it might have been more appropriate to select younger children. However, schoolchildren are more easily available for repeated investigations. We concentrated on representatives of the youngest and the oldest students. Their diet consisted primarily of porridge made from maize and "kaffir corn" (*Sorghum vulgar*) supplemented with beans and "morogo" (wild spinaches) and citrus fruits in season. Because of the high cost, pork or beef was rarely consumed, but most families kept chickens and ate one or two a week.

Fecal samples. Over a 15-month period fecal specimens were collected five times: twice in winter, and once each in autumn, spring, and summer. Children positive for *C. fetus* subsp. *jejuni* at any time were examined monthly for a further 3 months. The consistency of the stools was noted, and within 20 min of voiding, the stools were processed as follows.

Isolation of *C. fetus* subsp. *jejuni*. Skirrow's selective medium (10) was streaked with feces. The filtration technique was also employed for the isolation of *C. fetus* subsp. *jejuni* (5). About 1 to 2 g of the sample was placed in 6.0 ml of phosphate-buffered saline (pH 7.2), agitated on a Vortex shaker, and centrifuged for 5 min at 600 × *g*. The supernatant fluid was passed through a prefilter followed by a 0.65-μm pore size sterile membrane filter (Millipore Corp.); 0.1 to 0.2 ml of the filtrate was spread over the surface of a 10% horse blood agar plate (Columbia agar base, Oxoid Ltd.). The plates were stacked in stainless steel containers, which were flushed with a gas mixture consisting of 5% O_2, 10% CO_2, and 85% N_2, taped, and transported to the laboratory in Johannesburg. The inoculated media were incubated for up to 5 days in an atmosphere of 5 to 10% CO_2 in air; the blood agar

plates were incubated at 37°C, and the selective plates were incubated at 42°C.

Colonies which morphologically resembled *C. fetus* subsp. *jejuni* were subcultured for further examination. Isolates were identified (7) and tested for antibiotic sensitivity as previously described (5).

Salmonellae, shigellae, and parasites. Desoxycholate (BBL Microbiology Systems), MacConkey agar, and selenite F enrichment media were seeded with fecal material, transported to the laboratory, and incubated overnight. The next morning the enrichment cultures were streaked on fresh Desoxycholate agar plates. Non-lactose-fermenting colonies were tested biochemically, and those conforming to salmonellae were serotyped. Shigellae were classified according to their group antigen.

For parasitological investigation, stools were centrifuged and examined by the merthiolate-iodine-formaldehyde method (1).

RESULTS

Some children left school before the end of the survey, and others were absent on the day of collection of specimens, reducing the number of children present at all five collections to 73 (Table 1). Of these, 46 were 6 to 8 years old, and 27 were 13 to 16 years old.

Campylobacteriosis. *C. fetus* subsp. *jejuni* was isolated from nine children, three females and six males (Table 1). Children positive for *C. fetus* subsp. *jejuni* were followed up monthly for another 3 months. Table 1 shows that eight of the infections were observed in the 46 children aged 6 to 8 years. The organism was isolated intermittently from 10.9% for at least 9 months and from 4.1% for more than 1 year. The only infected subject among the 27 older children (3.7%) was positive for *C. fetus* subsp. *jejuni* for more than a year. Two of the three long-term-infected children excreted the organism at the time the study was terminated.

The prevalence of the infection was 8.2% in summer versus 3 to 5% in winter (Table 1).

The nine subjects from whom *C. fetus* subsp. *jejuni* was isolated were positive on 24 occasions (Table 1). All isolates were recovered by filtration techniques, but only 7 of 24 grew in primary culture on Skirrow's medium. However, in subcultures the selective medium supported growth of all 24 isolates.

All isolates displaying the typical morphology of *C. fetus* were identified as *C. fetus* subsp. *jejuni*. Eighteen of the isolates grew well at 42°C, and seven grew poorly. None grew microaerophilically at 25°C. All were susceptible to nalidixic acid (30 μg/disk).

In an attempt to differentiate between carriers and reinfected individuals, the isolates were further characterized. All were H_2S positive (0.02% cysteine hydrochloride), oxidase positive, and sensitive to NaCl (3.5%); all but one synthesized catalase. In contrast to the findings by Holdeman et al. (7), some of our isolates reduced nitrates; others did not. The reliability of the latter reaction as a marker for *C. fetus* subsp. *jejuni* was not determined. The antibiograms were remarkably uniform and virtually identical to those previously described (5). Only streptomycin seemed to have potential as an antibiotic marker. Of 19 isolates (5 died before they could be tested), 17 showed a minimal inhibitory concentration of 0.6 μg/ml (range, 0.25 to 2 μg/ml), whereas two strains gave minimal inhibitory concentration values of 4 and 8 μg/ml with correspondingly small zones around the disks (4). One of the resistant strains was isolated from child no. 18, who later yielded three organisms of average streptomycin susceptibility. The most resistant strain was isolated on one occasion only—from child no. 54.

TABLE 1. *Seasonal incidence of C. fetus subsp. jejuni and persistence of infection in 73 children*

Child no.	Age (yr)	Sex	Presence or absence of *C. fetus* subsp. *jejuni*								Persistence of infection (months)
			April 1978 (fall)	July 1978 (winter)	October 1978 (spring)	January 1979 (summer)	July 1979 (winter)	September 1979 (spring)	October 1979 (spring)	November 1979 (spring)	
18	6	F	−	+	+	+	−	−	+	+	≥16
38	6	M	−	+	−	+	+	+	+	−	≥16
96	13	M	−	+	−	+	−	−	+	+	≥16
54	8	M	−	−	−	+	−	−	−	+	≥11
55	8	M	−	−	−	+	−	−	+	−	≥10
28	6	M	−	−	+	−	+	−	−	−	≥9
31	6	M	−	+	+	−	−	Left village			≥3
19	6	F	−	−	−	+	−		−	−	0
22	6	F	−	−	+	−	−	−	−	−	0
% Positive[a]			0%	5.5%	5.5%	8.2%	2.7%				

[a] Percentage of 73 children positive for *C. fetus* subsp. *jejuni*.

Salmonellae, shigellae, and parasites. Samples from 6 of the 73 children yielded growth of salmonellae, and 6 yielded growth of shigellae. None of these pathogens were isolated from individuals with fecal *C. fetus* subsp. *jejuni* infections. Approximately 6.5% of the children were infected with *Giardia lamblia. Hymenolepis nana, Entamoeba histolytica,* and *Ascaris lumbricoides* were observed on occasions. All children were asymptomatic.

DISCUSSION

Reinfection versus carriers. From available data it cannot be determined whether all seven individuals from whom *C. fetus* subsp. *jejuni* was isolated repeatedly (Table 1) had become reinfected on one or more occasions or whether they were carriers. Neither biochemical reactions nor antibiograms provided reliable bacteriological markers. Serotyping and bacteriophage typing, both in their developing stages, hold out hope for the future.

Epidemiological data tend to suggest that the seven long-term-infected individuals were carriers: (i) in a homogenous population one would expect that multiple infections were randomly distributed and not confined to a small select group; (ii) in developed countries the organism is ubiquitous, suggesting that in underdeveloped countries it would be hard not to be constantly exposed to *C. fetus* subsp. *jejuni*; and (iii) intermittent excretion of pathogens is well known in typhoid carriers. It is possible that the observed intermittency reflects the limit of sensitivity of the techniques for detecting *C. fetus* subsp. *jejuni.* Recovery of *C. fetus* subsp. *jejuni* by the filtration technique and by our selective media requires the presence of approximately 5,000 and 1,000 organisms per g of feces, respectively (unpublished observations). Thus, samples with fewer than 1,000 organisms per g of feces will yield negative results.

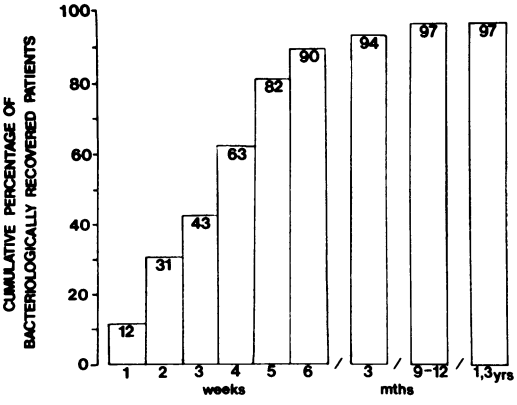

FIG. 1. *Proposed duration of spontaneous bacteriological recovery from C. fetus subsp. jejuni infections among Tlaseng children based on the recovery rate reported by Karmali and Fleming (8) and our findings.*

Risk of long-term colonization. On the assumption that the long-term-infected individuals were indeed carriers, we may calculate the minimum risk of an acute infection, clinical or subclinical, resulting in long-term colonization. From previous observations it is reasonable to assume that most children from underdeveloped countries have suffered at least one *C. fetus* subsp. *jejuni* infection before they start school (2, 5). Among the 46 children 6 to 8 years old, we observed five (10.9%) who were infected for at least 9 months and two (4.3%) who were infected for at least 16 months. The carrier rate could be higher, because of failure to detect some mild, intermittent excreters and the possibility of a less than 100% exposure to *C. fetus* early in life would tend to increase the observed risk of becoming a carrier. It is noteworthy that among the 27 older children there was only one (3.7%) long-term-infected individual, suggesting that spontaneous bacteriological cure or resistance to reinfection occurs with time. This is consistent with the rarity of disseminated campylobacteriosis due to *C. fetus* subsp. *jejuni* in elderly, debilitated individuals in whom the predominating organism is *Campylobacter fetus* subsp. *intestinalis* (3).

Our findings appear to be at variance with the observations of Karmali and Fleming (8). They found that 50% of 24 untreated children with acute campylobacter enteritis ceased to excrete the organisms within 3 weeks of onset, and 100% were bacteriologically cured within 7 weeks. Several factors tend to reduce the differences between the surveys. First, in a group of 24 children one could only expect 1 or 2 long-term-colonized individuals. Second, as stated above, intermittent excreters are easily missed. Third, all isolates of *C. fetus* subsp. *jejuni* in this study were recovered by filtration technique, whereas 17 of 24 failed to grow in primary culture on Skirrow's medium. Thus, differences in findings could be attributable to differences in techniques. It is conceivable, then, that the natural history of campylobacter enteritis in South African populations is best appreciated by taking both studies into account. For the purpose of the calculation it is assumed that all 73 children in our study experienced an infection with *C. fetus* subsp. *jejuni* before starting school. In that case, at least 7 of 73 (9.6%) of the infected individuals would remain infected for more than 3 months (Table 1). The remaining 91% were either per-

sistently negative or children from whom *C. fetus* subsp. *jejuni* was isolated only once. It may be assumed that they achieved bacteriological clearance as described by Karmali and Fleming (8). The results of this computation are shown in Fig. 1, which graphically expresses the spontaneous bacteriological recovery as it would appear to occur under the epidemiological conditions prevailing in South Africa. It suggests that at least 3% of untreated children carry the organisms for a year or more. Furthermore, if less than 100% of the children were infected with *C. fetus* subsp. *jejuni* before the survey, the probability of an infection resulting in a long-term-carrier state would be still higher.

Alternative explanations are possible. First, the Canadian and the South African populations live under vastly different conditions. Second, infecting strains may differ in their ability to colonize the gut. And, third, the immune response may depend on the severity of the primary infection: clinical or subclinical. The Canadian children suffered clinically manifest *C. fetus* subsp. *jejuni* enteritis (8), whereas the South African children had no recent history of diarrhea.

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