Isolation of *Campylobacter fetus* subsp. *jejuni* from Bangladeshi Children

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To determine the prevalence of infection with *Campylobacter fetus* subsp. *jejuni* in Bangladesh, culture surveys were conducted among three populations. In Dacca, *Campylobacter* was isolated from 5.2% of 97 individuals with clinical dysentery and from 12.3% of 204 patients with only diarrhea. This difference may have resulted from a greater representation of young children in the second group. *Campylobacter* was isolated from 17.7% of the 141 healthy village children aged 1 to 5.5 years and from 38.8% of the 1-year-old children. More infected children (48%) had a history of recent diarrheal illness than did a group of matched controls (20%; *P* = 0.016). These findings suggest that campylobacter infection is common for Bangladeshi children. However, this organism may not cause diarrheal illness in all instances in which it is isolated.

Studies in Europe, Canada, Australia, and the United States have shown that *Campylobacter fetus* subsp. *jejuni* is a common human enteric pathogen (1, 5, 11, 13, 15). Because in the developed countries *Campylobacter* has usually been isolated from individuals who sought medical attention because of diarrhea, the clinical spectrum of campylobacter enteritis has been defined from studies of these populations (1, 5, 13).

Several studies from developing countries have documented the isolation of *Campylobacter* from patients with diarrhea (4, 6, 12); however, its role as a pathogen has not been well established. Recently, Bokkenheuser and colleagues showed that *Campylobacter* was frequently isolated from healthy children in South Africa (3). These findings suggest that some aspects of the epidemiology of campylobacter infection may differ in the developed and the developing countries.

Bangladesh is a developing country where enteric diseases, including cholera, are endemic (10); morbidity and mortality due to diarrheal illness is common among children. If the prevalence of infection with a known enteric pathogen such as *Campylobacter* can be defined, it might be possible to institute specific measures for better control of diarrheal illness. An earlier survey in Dacca found *Campylobacter*-like organisms in the stools of 8% of patients with bloody diarrhea (R. Wilson and M. I. Huq, unpublished data). In this study we utilized a method for isolating *Campylobacter* from clinical specimens suitable for developing countries and then surveyed three different populations to determine the prevalence of campylobacter infection.

MATERIALS AND METHODS

Populations studied. We studied the rates at which *C. fetus* subsp. *jejuni* was isolated from members of three population samples (groups A through C) in Bangladesh whose clinical characteristics were different. Group A consisted of a 2% random sample of patients seen at the clinic of the International Centre for Diarrheal Disease Research, Bangladesh, in Dacca from 20 February to 20 March, 1980. Since the clinic is known in the community as a “diarrhea hospital,” virtually all clinic patients had diarrheal symptoms.

Group B consisted of individuals who came to the clinic during the same period but who stated in a screening interview that they had had both blood and mucus in their stools (clinical dysentery).

Group C consisted of 141 healthy children, 1 to 5.5 years of age, from two villages in the Matlab census area, whose 111 randomly picked families were enrolled in an ongoing prospective study (R. E. Black, M. H. Merson, K. H. Brown, A. S. M. M. Rahman, M. Yunus, A. R. M. A. Alim, V. A. Lottidge, R. H. Yolken, and G. T. Curlin, Abstr. 13th Joint Conf. Viral Dis. U.S.-Jpn. Coop. Med. Sci. Program, Atlanta, Ga., 1979, p. 31) of the effects of diarrheal illness on growth and development. In the growth and development study, rectal swabs were obtained from these children both whenever they had an episode of diarrheal illness and monthly whether they were sick or well. In the current study we cultured the March 1980 (monthly) swabs for *Campylobacter*. Past experience with that population had shown that at any one time up to 20% of the children had diarrheal symptoms (Black et al.,
whether
with
42°C (W.
ate
tericin
sample
all
Med. Sci.
systemic
symptoms.
Microbiological methods. The primary plating
medium we used was Campy-BAP (brucella agar base
[BBL Microbiology Systems, Cockeysville, Md.] with
5% sheep erythrocytes and the following antimicro-
cial concentrations per liter: vancomycin, 10 mg;
trimethoprim, 5 mg; polymyxin B, 2,500 IU; amphi-
tericin B, 2 mg; and cephalexin, 15 mg [1]). All plates
were incubated at 42°C. Because an appropriate bot-
tled gas mixture and vacuum jars were not available,
all incubations were done in candle jars to reduce the
ambient oxygen concentration. Other investigations
have shown that using candle jars is as effective as
using an atmosphere with 6% oxygen for primary
isolation of C. fetus subsp. jejuni when incubation is
at 42°C (W. L. Wang and N. W. Luechtfeld, personal
communication).
Rectal swabs or stool specimens collected at Dacca
were inoculated immediately to the plating medium.
Rectal swabs obtained from the village children were
transported to the laboratory in Cary-Blair medium
(J. G. Wells, T. J. Barrett, and H. E. Sours, Abstr.
and inoculated within 4 h of being obtained. Prelimi-
nary studies have shown that Cary-Blair medium is
effective for transporting fecal specimens containing
Campylobacter at ambient temperatures for 6 h or
more (17).
Plates were read at 48 h, and colonies with charac-
teristic morphology were Gram stained and tested for
motility and oxidase and catalase production. Orga-
nisms that were oxidase- and catalase-positive, motile,
gram-negative vibrios were transported to the Center
for Disease Control in Wang’s medium (brucella broth
[BBL] with 0.5% agar and 10% defibrinated sheep
blood) (16) or as lyophils. Identification of organisms
as C. fetus subsp. jejuni was done according to stan-
dard criteria (14). Isolation of Salmonella and Shigella
from specimens from the village children were done with
MacConkey and salmonella-shigella agars (7).

RESULTS
Campylobacter-like organisms were isolated from
the feces of 55 of the 442 Bangladeshis from whom specimens were obtained for culture in these studies. All 43 viable strains received by the Center for Disease Control were identified as C. fetus subsp. jejuni according to the following characteristics: typical colonial morphology on the Campy-BAP medium; gram-negative, curved, motile rods; positivity for oxidase and
catalase; growth in brucella broth at 37 and 42°C
but not at 25°C; no growth in 3.5 and 6.5% NaCl;
multiplication in 1% glycine; H2S production in
Kligler medium with lead acetate paper; and
sensitivity to nalidixic acid.
Clinic populations. C. fetus subsp. jejuni
was isolated from 12.3% of the members of group
A (Table 1), with the highest rate among chil-
dren (21%) being for those less than 1 year of
age. Children under 5 years of age constituted
66% of this population. Although the isolation
rate in 110 children under 3 years of age (15.5%) was
higher than in individuals 3 to 66 years of age
(8.5%), the differences were not statistically
significant. For the entire group there were no
significant differences in the isolation rate ac-
cording to sex.
Campylobacter was isolated from 5.2% (Table
1) of the patients with bloody diarrhea (group
B) seen at the clinic during the same time period.
Children under 5 years of age constituted 37% of
this population. Only 1 of 47 (2%) persons over
the age of 10 was positive for Campylobacter.
Village children. C. fetus subsp. jejuni was
isolated from 17.7% of 141 village children (Table
2); the rates for males (17.3%) and females
(18.3%) were similar. In contrast, Shigella was
isolated from only three children (2%), and Sal-
onella was not isolated at all. The highest
isolation rate was for children 12 to 23 months
of age (38.9%), significantly higher than the iso-
lation rate for the older children (χ2 = 4.8; P =
0.029).
There was no spatial clustering associated
with infection; children infected with Campylo-
bacter resided in 21.6% of the 111 households
from which cultures were obtained. In 85 of

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>No. of patients tested</th>
<th>Isolation rate/100 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>52</td>
<td>21.2</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>9.5</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>12.5</td>
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<tr>
<td>3</td>
<td>9</td>
<td>11.1</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>5-9</td>
<td>17</td>
<td>5.9</td>
</tr>
<tr>
<td>10-19</td>
<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>20-39</td>
<td>30</td>
<td>3.3</td>
</tr>
<tr>
<td>40+</td>
<td>15</td>
<td>26.7</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>12.3</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Age group (yr)</th>
<th>No. of patients tested</th>
<th>Isolation rate/100 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1-4</td>
<td>34</td>
<td>5.9</td>
</tr>
<tr>
<td>5-9</td>
<td>14</td>
<td>14.3</td>
</tr>
<tr>
<td>10-19</td>
<td>14</td>
<td>7.1</td>
</tr>
<tr>
<td>20-79</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>5.2</td>
</tr>
</tbody>
</table>

\(^a\) ICDDR, B, International Centre for Diarrheal Disease Research, Bangladesh.
these households in which specimens for culture were obtained from only one child, 16.5% of the children were infected; in 36.4% of 22 of these households in which specimens for culture were obtained from two children, at least one child was infected; and in 50% of 4 of these households in which specimens for culture were obtained from three children, at least one child was infected. In only 1 of the 26 households (3.8%) in which specimens for culture were obtained from two or more siblings were there two infected children. Similarly, of 12 children who lived in the same household with an infected sibling and from whom specimens for culture were obtained, only one specimen was positive (8.3%).

Each of the 25 children from whom Campylobacter was isolated was matched with 2 culture-negative controls who lived in the same village, but not in the same household, and were sex and age (within 6 months) matched. Records of interviews with mothers of these children were reviewed to determine whether any diarrheal episodes had been noted in the 30 days before the culture was prepared. A diarrheal episode was defined as four or more loose stools a day, with or without abdominal pain and fever, for at least 1 day (1). Twelve of the children from whom Campylobacter had been isolated (48%) had had symptoms within 1 month before the culture (Table 3), as had 10 (20%) of the controls. Although not all children infected with Campylobacter had a recent history of diarrhea, significantly more of them did than controls \((P = 0.016, \text{two-tailed Fisher's exact test})\) (9).

### TABLE 2. Isolation of C. fetus subsp. jejuni from village children, Matlab census area (group C), March 1980

<table>
<thead>
<tr>
<th>Age group (mo)</th>
<th>No. of children tested</th>
<th>Isolation rate/100 children</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-23</td>
<td>18</td>
<td>38.9</td>
</tr>
<tr>
<td>24-35</td>
<td>37</td>
<td>16.2</td>
</tr>
<tr>
<td>36-47</td>
<td>46</td>
<td>19.6</td>
</tr>
<tr>
<td>48-65</td>
<td>40</td>
<td>7.5</td>
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<tr>
<td>Total</td>
<td>141</td>
<td>17.7</td>
</tr>
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</table>

### TABLE 3. Comparison of the frequency of diarrheal episodes in children infected with Campylobacter and in uninfected children

<table>
<thead>
<tr>
<th>Timing of episode</th>
<th>No. of children with a diarrheal episode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture positive ((n = 25))</td>
</tr>
<tr>
<td>1-7 days preceding culture</td>
<td>5</td>
</tr>
<tr>
<td>8-14 days preceding culture</td>
<td>3</td>
</tr>
<tr>
<td>15-30 days preceding culture</td>
<td>10</td>
</tr>
<tr>
<td>Any time in preceding mo</td>
<td>12 (48%)*</td>
</tr>
</tbody>
</table>

*A child who had diarrhea during more than one time period was counted only once.

### DISCUSSION

This study shows that C. fetus subsp. jejuni can be isolated from clinical specimens without using apparatus to reduce the oxygen level to 5%. Although the candle jar may not provide the optimal atmosphere for all strains of C. fetus subsp. jejuni, it can be used in field settings and seems to provide a sufficiently low level of oxygen for the frequent isolation of Campylobacter from clinical specimens. Whether a higher isolation rate would be found with an oxygen level of 5%, direct plating, or refrigerated carrier media will require further study.

C. fetus subsp. jejuni has been isolated from individuals from all parts of the globe (1, 3, 6, 11, 12, 15). Most of the epidemiological investigations of campylobacter infections have been in the developed countries, and most Campylobacter isolates have been obtained from patients with diarrhea or from asymptomatic individuals exposed to a common vehicle (1, 5, 10). Studies of patients presenting to hospitals complaining of diarrheal illness indicate that Campylobacter has been isolated about as frequently as Salmonella or Shigella (1, 11, 13, 15). Isolation of C. fetus subsp. jejuni from healthy individuals in developed countries has been infrequent (2, 13, 15), although Lauwers and colleagues isolated Campylobacter from 1.3% of a group of "asymptomatic" children tested in Brussels (8). However, most of these children were newly arrived immigrants from North Africa, living in poor conditions, and no information was obtained about their having had recent episodes of diarrhea.

Few data are available on the incidence of Campylobacter isolates from healthy populations in the developing world (4, 6, 12). Several reports have indicated that Campylobacter was isolated from patients with diarrhea, but there have been few controls. In one study (6) Demol and Bosmans found that 9% of children infected with measles were also infected with Campylobacter. Recently, Bokkenheuser and colleagues studied the summertime rates of isolation of Campylobacter from black South African children living in a rural area (3). C. fetus subsp. jejuni was isolated from 35% of 78 children with diarrhea and from 10 (16%) of 63 children without diarrhea. Campylobacter was isolated from 44% of children between the ages of 9 and 24 months who did not have diarrhea.

In our study of village children in Bangladesh during the dry season, Campylobacter was frequently isolated, with an infection rate for village...
children 12 to 23 months of age of nearly 40%. If this rate is typical for the whole year, the incidence of infection must be high: if a child carried the organism for 1 month after being infected, the average child would have five infections a year; if it were carried for 3 months, the same child would have one or two infections a year. The lack of multiple infections in a household suggests that there is relatively little person-to-person transmission. Further studies are needed to test these hypotheses.

Children in rural Bangladesh commonly have diarrhea, although it is not usually severe enough to necessitate a visit to the clinic (Black et al., Abstr. 13th Joint Conf. Viral Dis. U.S.-Jpn. Coop. Med. Sci. Program, 1979). Fewer than half of the Campylobacter-positive patients in our study had a history of diarrhea within 1 month of the time specimens for culture were obtained, which suggests that infection is frequently asymptomatic or that the organism is carried by the patients for several months after they are infected. The study of culture-positive and culture-negative children showed a significant association between having had diarrhea and isolation of Campylobacter. These data are compatible with the hypothesis that infection with C. fetus subsp. jejuni may be associated with disease in some instances in Bangladesh as it is in developed countries.

The lower rate at which Campylobacter was isolated from patients in Dacca who had diarrhea than from the village children may in part result from the different age distributions of the three populations. Age appears to be a significant variable affecting the isolation rates for all three groups.

The high prevalence of campylobacter infection in a population of village children suggests that in Bangladesh these organisms may not have been the etiological agents for diarrheal illness whenever they were isolated. Further studies are needed to assess the pathogenicity of isolates from symptomatic and asymptomatic individuals and to determine the natural history of, and immunity to, campylobacter infection in Bangladesh.

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LITERATURE CITED


