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SALMONELLOSIS AT RURAL AND URBAN CLINICS IN BANGLADESH

EPIDEMIOLOGIC AND CLINICAL CHARACTERISTICS

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The authors studied the frequency of diarrheal illness associated with non-*typhi Salmonella* at two clinics in Bangladesh for the years 1977–1979. Non-*typhi* salmonellae were isolated from 0.29% of fecal specimens or rectal swabs in an urban area and 0.26% of similar specimens in a rural area; the frequency of isolations peaked in the summer months. Isolations of *Shigella* and *Vibrio cholerae* were much more common than *Salmonella*. Only two of 50 *Salmonella* isolates were resistant to more than one antibiotic. None of 13 isolates tested produced an enterotoxin. *S. java* and *S. virchow* accounted for 64% of all the isolates. Patients with diarrheal illness associated with isolation of *Salmonella* frequently had vomiting (88%), watery diarrhea (78%), abdominal pain (61%), and fever (39%), but the clinical features of the illnesses and the socioeconomic backgrounds of the patients could not be distinguished from those of matched controls who were attending the same clinic. The infrequency of *Salmonella* infection in an area where several other bacterial and viral enteric diseases are hyperendemic requires further investigation.

antibiotics; developing countries; diarrhea; enterotoxins; *Salmonella*; serotyping

Non-typhoid salmonellosis continues to be a significant public health problem in the United States and Europe (1, 2), while typhoid and paratyphoid are of far less significance. Animals are recognized to be the major reservoir for non-*typhi* salmonellae; modern methods of animal husbandry, food production, and food handling may encourage the transmission of these organisms from animal products to man (3).

In the developing countries, however, *Salmonella typhi* and *S. paratyphi* A and B often cause major public health problems. Humans are the reservoir for these agents, and infection is prevalent in areas where standards of hygiene are low. Assessment of the significance of non-*typhi Salmonella* infections in the developing countries has been limited (4–9). In Bangladesh, a developing country where cholera, shigellosis, enterotoxigenic *Escherichia coli*, and rotavirus infections are hyperendemic (10–12), we sought to learn more about the magnitude and character of non-typhoid salmonellosis and to examine the clinical and epidemiologic features of non-*typhi Salmonella* infections.

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Abbreviation: ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh.

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Materials and methods

Populations studied

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has treatment centers for the diagnosis and treatment of patients with diarrheal illnesses in Dacca City and Matlab Thana, about 48 km (30 mi) from Dacca. The Dacca clinic serves a population that is predominantly urban, and the Matlab clinic serves a rural population.

Epidemiologic methods

We reviewed microbiology laboratory records at both the Dacca and Matlab clinics for the years 1975–1979 to determine the number of fecal cultures performed and the number of isolations of non-*typhi Salmonella, Shigella,* and *Vibrio cholerae.* Only rarely were multiple specimens submitted from the same patient.

We reviewed the clinical records of the patients at Matlab from whom non-*typhi Salmonella* was isolated. To determine whether the age and sex distribution of the *Salmonella*-infected patients (cases) was similar to patients with a diarrheal illness who were not infected with *Salmonella* (control group), we matched each case with two controls whose registration numbers were immediately before and after those of the cases.

Most patients cultured at the Matlab treatment center came from a surveillance area where all persons had an identification number based on the censuses of 1974 and 1979 (13). For each of 170,000 individuals in the surveillance area, the census records contained information on family, bari (neighborhood) and village size, religion, occupation and educational level of the head of the family, sources of income, size and nature of housing, water supply, and family possessions. Before 1978, some of the patients who were cultured resided outside the surveillance area.

For an assessment of the distinctive clinical features and the socioeconomic background of the 70 patients with *Salmonella* infection, we selected only those for whom complete clinical and census records were available. Patients who were infected with *Salmonella* and any other etiologic agents (such as *V. cholerae, Shigella,* enterotoxigenic *E. coli,* and rotavirus) were not included in this part of the study. Of the 70 patients known to be infected with non-typhi *Salmonella,* 47 were excluded because census or clinical records were not available or were incomplete or stools were not tested for rotavirus and enterotoxigenic *E. coli,* and five were excluded because of co-infection with another agent. For each of the remaining 18 patients infected with *Salmonella*, we randomly selected from the registration books two other controls who were matched for age (within one year for those aged under 5 years, two years for those aged 5–15 years, and five years for those aged over 15 years), sex, and month of presentation to the clinic. In a matched triplet analysis, we compared clinical records and census data for the patients and the control group using a chi-square statistic with one degree of freedom (14).

Microbiology methods

Procedures for isolating *Salmonella* at the two microbiology laboratories were uniform and standard (15). About 90 per cent of the fecal specimens received for culture were rectal swabs. A swab from a stool specimen, or a rectal swab, was inoculated onto MacConkey medium (Difco Co., Detroit, MI), and *Salmonella-Shigella* (S-S) agar (Difco), and into selenite broth (Difco). After 18–24 hours incubation at 37 C, the selenite was subcultured to both the MacConkey's and S-S media. All plates were incubated at 37 C for 24 hours. Colonies that were non-lactose fermenting were subcultured on Kligers' iron agar (KIA) (Difco) and motility

indole urea (MIU) (Difco) media. Those organisms that had an acid-gas reaction and were lactose-negative in KIA and were motile but indole- and urea-negative in MIU were considered to be salmonellae. Identification was confirmed using polyvalent antisera (Burroughs Wellcome Co., London, England) and grouping was with group-specific antisera (Burroughs Wellcome).

To assess the sensitivity of these methods under field conditions, five stool suspensions were seeded with log dilutions of Group B *Salmonella* producing final concentrations of 10^2-10^6 *Salmonella*/ml. One swab from each of the five suspensions was inoculated, and the swabs were sent to the diagnostic laboratory as routine specimens for culture.

All *Salmonella* isolates stored on KIA slants or as lyophils in the ICDDR,B culture collection from Matlab for the period 1977–1979, and a random selection of those from Dacca during the same period, were reconstituted and brought to the Centers for Disease Control (CDC) for serotyping (15). Isolates were tested at CDC for susceptibility to antimicrobial agents using the method of Kirby and Bauer (16). Thirteen isolates obtained from feces of patients with well characterized clinical illness were tested for the presence of a heat-labile enterotoxin using the Y-1 adrenal cell assay (17), for heat-stable enterotoxin using the infant mouse model (18), and for invasiveness using the Sereny test (19).

Results

Isolation of Salmonella *in Dacca*

In the three-year period from October 1977–September 1979, 66,342 cultures of fecal specimens were taken in the laboratory at Dacca; each year the number of cultures taken decreased due to a decrease in available resources and a subsequent curtailment of the catchment area (table 1). Non-*typhi Salmonella* was

TABLE 1

Isolations of Vibrio cholerae, Shigella, *and* Salmonella *from patients with diarrheal illnesses, Bangladesh, October 1976–September 1979*

Place and year*	Total cultures	*Vibrio cholerae*		*Shigella*		Non-*typhi Salmonella*					Total non-*typhi Salmonella*		*S. typhi*†
		No.	%	No.	%	A	B	C_1	C_2	D	E	No.	%	
Dacca														
1977	31,229	1454	4.7	3648	11.7	6	32	32	21	1	16	108	0.34	66
1978	20,559	684	3.3	1295	6.3	7	17	10	8	0	9	51	0.25	73
1979	14,554	1133	7.8	730	5.0	4	16	5	3	0	3	31	0.21	75
Total	66,342	3271	4.9	5673	8.6	17	65	47	32	1	28	190	0.29	214
Matlab														
1977	15,867	2038	12.8	736	4.6	2	14	22	7	2	1	48	0.30	7
1978	7120	978	13.7	432	6.1	0	2	10	2	1	1	16	0.22	2
1979	4278	572	13.4	354	8.3	0	2	1	3	0	0	6	0.14	3
Total	27,265	3588	13.2	1522	5.6	2	18	33	12	3	2	70	0.26	12

* Fiscal year (October 1 to September 30).
† Isolates from blood and stool.

isolated from 0.29 per cent of specimens; in contrast, *Shigella* species were isolated from 8.6 per cent of specimens, and *V. cholerae* from 4.9 per cent of specimens. Group B salmonellae (34 per cent) were the most frequently isolated followed by Group C_1 (25 per cent). *S. typhi* was isolated more frequently than all the non-*typhi* salmonellae combined; however, most of these isolations were from blood cultures.

Figure 1 shows the number of non-*typhi Salmonella* isolates in Dacca and Matlab by month for a five-year period. No seasonal variability is readily apparent from this figure; however, 47 per cent of the isolates in Dacca were obtained during the hot and wet months of May through August. The total number of specimens submitted for culture each month in Dacca could not be determined. A peak in isolations was observed from May through September 1977; 29 of the 67 isolates during that period were Group B, and 19 were Group C_1. In July 1977, 11 isolates were Group B, the largest clustering of a single serogroup during the five years of surveillance.

Isolation of Salmonella *in Matlab*

For the period from October 1977–September 1979, 27,265 cultures were obtained at Matlab (table 1). The serial decrease in the annual number of cultures done was due to ceasing culturing of non-surveillance area patients and a further curtailment of the surveillance population. As in Dacca, the rate of isolation of non-*typhi Salmonella* species was significantly lower than the rates of isolation of *Shigella* and *V. cholerae*. As shown in figure 1, the monthly number of isolates peaked from May through August 1977 in Matlab as well as in Dacca. Most serogroups were represented in these peaks, but, as in Dacca, the majority of the isolates were Groups B and C_1. Rainfall was within the usual range for that time of year and the riverine waters did not crest until late August, which is also usual. In the entire three-year period in Matlab, Group C_1 was most frequently isolated (47

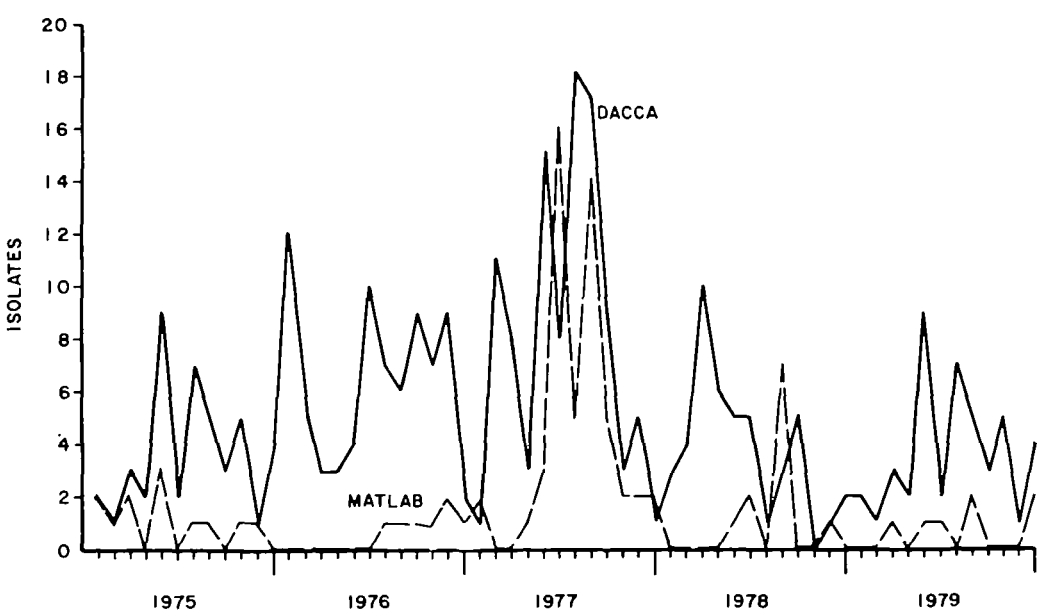

FIGURE 1. Isolates of *Salmonella* from patients presenting to the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) clinics in Dacca and Matlab, Bangladesh, by month, 1975–1979.

per cent) followed by Group B (26 per cent). Blood cultures were not done in Matlab; fecal shedding of *S. typhi* was uncommon in this patient population.

The majority of the salmonellae were isolated in Matlab from May through August (74 per cent of the total). This was not an artifact of increased culturing, since the rates of isolation (in per cent) per cultures obtained also peaked during those months (figure 2). The isolation rate for *Salmonella* showed a seasonal peak as did the rate for *V. cholerae,* but not the rate for *Shigella.*

Laboratory results

The microbiology laboratory in Dacca isolated *Salmonella* from four of the five seeded stool specimens in the blind trial, including the specimen which contained 10^2 organisms/g, but it did not isolate *Salmonella* from the specimen with 10^3 organisms/g. Each isolate was correctly identified as being Group B.

In all, 63 isolates of *Salmonella* were recovered from the culture collections of ICDDR,B. When the specimens were analyzed at CDC, however, 11 were no longer viable and two were found not to be *Salmonella.* In both Matlab and Dacca, *S. java* was the most common serotype, followed by *S. virchow;* these two serotypes together accounted for 64 per cent of the isolates (table 2). Although from May to September 1977 the number of *Salmonella* isolates in both Dacca and Matlab increased significantly (figure 1), the same serotypes did not predominate in the two locations. Of 10 strains from Dacca during that period, eight were *S.*

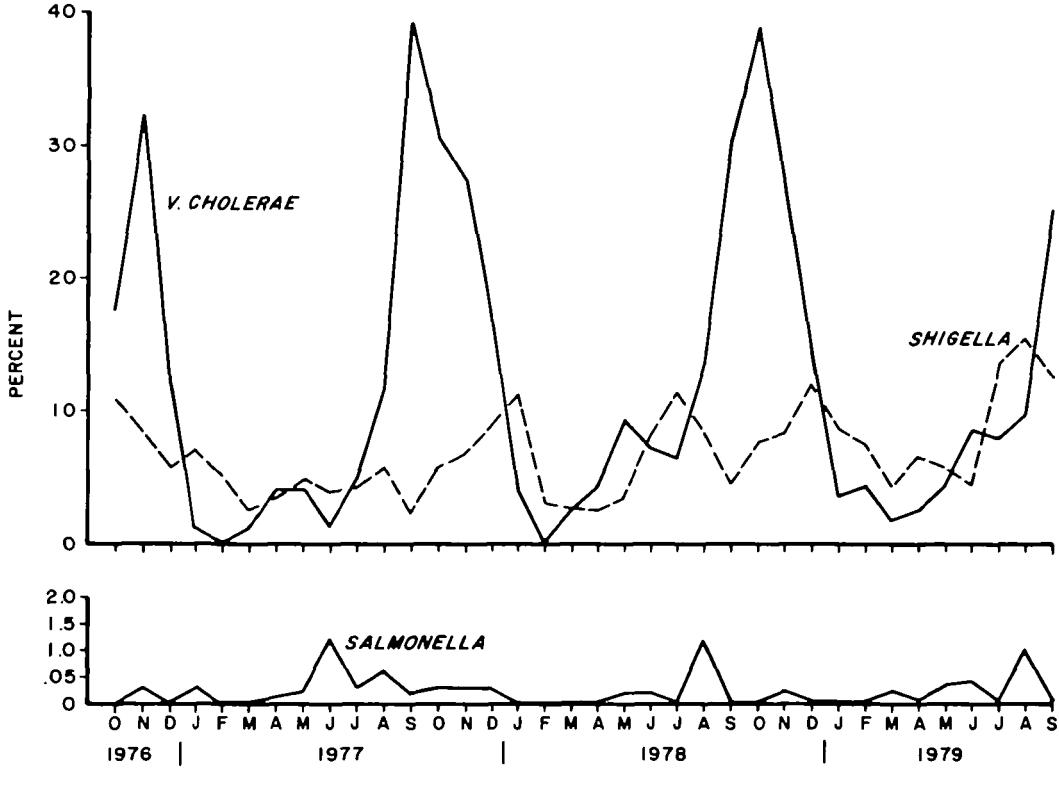

FIGURE 2. Isolation rate (per cent) for *Vibrio cholerae, Shigella,* and *Salmonella* from patients presenting to the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) clinic in Matlab, Bangladesh, by month, October 1976–September 1979.

TABLE 2

*Serotypes of 50 non-*typhi Salmonella *strains isolated from patients in Dacca and Matlab, Bangladesh, 1977–1979*

Serotype (and serogroup)	No. of isolates		
	Dacca	Matlab	Total
S. java (B)	10	11	21
S. virchow (C_1)	4	7	11
S. weltevreden (E_1)	4	1	5
S. augustenborg (C_1)	0	4	4
S. paratyphi A (A)	4	0	4
S. litchfield (C_2)	0	3	3
S. stanley (B)	1	0	1
S. javiana (D)	1	0	1
Total	24	26	50

java, one was *S. virchow,* and one was *S. weltevreden;* in Matlab there were four *S. virchow,* three *S. augustenborg,* two *S. java,* and one *S. weltevreden.* All four isolates from a 1980 family cluster of diarrheal illness near Matlab were *S. java.*

Susceptibility testing of the 50 isolates showed a low frequency of resistance to antimicrobial agents. None of the isolates were resistant to tetracycline, kanamycin, gentamicin, chloramphenicol, nalidixic acid, nitrofurantoin, or colistin. Of four *S. java* isolates (8 per cent) which were resistant to ampicillin, one was also resistant to cephalothin. These *S. java* isolates were obtained from patients in Dacca during July and August 1977. Because of the spatial and temporal clustering of four isolates of the same serotype with the same unusual resistance to ampicillin, these *S. java* infections probably represent exposure to a common source. No other epidemiologic data about these cases are available. One isolate of *S. virchow* was resistant to both sulfathiazole and streptomycin. None of the 13 isolates tested, including six from patients with watery diarrhea, produced a heat-stable or heat-labile enterotoxin. None of the isolates were invasive in the Sereny test; no mild or equivocal changes were seen.

Clinical and epidemiologic features of salmonellosis in Matlab

Sixty-five *Salmonella*-infected patients seen at Matlab ranged in age from three months to 71 years (median 12 years), but were not significantly different from those of the randomly selected controls seen at the same clinic. Forty-one *Salmonella* patients (63 per cent) were males; the same sex distribution was seen in the control population.

The age- and sex-matched controls of the 18 patients infected with *Salmonella* were infected by a variety of pathogens. The following etiologic agents—*V. cholerae,* 7; *Shigella,* 7; non-O group 1 *V. cholerae,* 3; *Entamoeba histolytica,* 2; enterotoxigenic *E. coli,* 1; rotavirus, 1—were isolated from 19 of the 36 controls (53 per cent); however, only 19 had been tested for rotavirus and only four for enterotoxigenic *E. coli.* There were no significant differences between the patients infected with *Salmonella* and the controls in the frequency of watery (78 per cent vs. 72 per cent), bloody (6 per cent vs. 8 per cent), or mucoid diarrhea (39 per cent vs. 22 per cent), presence of vomiting (83 per cent vs. 81 per cent), abdominal pain (61 per cent vs. 44 per cent), and fever (39 per cent vs. 28 per cent).

The stools of only six patients infected with *Salmonella* were examined microscopically; all specimens had white blood cells, five had mucus, and three had red cells. The median duration of illness before coming to the clinic was 13 hours (range, 1–251 hours). A five-month-old girl with salmonellosis and dehydration died; all other patients were discharged from the clinic. The median duration of illness from onset of symptoms until discharge from the clinic was 56 hours (range, 13–263 hours).

There were no significant differences between the patient with salmonellosis and the matched controls for duration of stay at the clinic, total duration of illness, stool volume while at the clinic, stool volume per kilogram-patient weight, total fluids given, or fluids per kilogram. Despite the fact that the patients infected with *Salmonella* and the controls received equivalent amounts of fluid therapy, the two groups differed significantly in terms of how much weight they gained while at the clinic, presumably through rehydration. Most of the controls gained weight at the clinic (78 per cent), indicating that they had been fluid-depleted at the time of coming to the hospital, whereas only 47 per cent of patients infected with *Salmonella* gained weight ($\chi^2 = 3.7$, $p = 0.054$).

The clinical features of the seven control group patients who had cholera were substantially different from those in the patients with *Salmonella* infection. Significant dehydration was seen in 71 per cent of cholera cases but only 12 per cent of *Salmonella* cases ($p = 0.007$, Fisher's exact test, one-tailed). Rehydration increased body weight in 86 per cent of the cholera cases but in only 47 per cent of the salmonellosis cases.

The socioeconomic backgrounds of the 18 patients with salmonellosis were compared with those from the same matched 36 patients in the control group. There were no significant differences between the cases and the matched controls in the size of their dwellings and type of structure, and number of square feet of dwelling space per capita; family, bari, or village size; religion; presence of, or proximity to, a latrine at home; source of water for drinking, bathing, or cooking; and family wealth or educational level. One difference noted was that fewer patients infected with *Salmonella* (12/18) washed their hands after defecation than did matched controls (32/35) ($p = 0.06$).

Discussion

Salmonellosis does not appear to be a common illness in either of the two areas in Bangladesh that we studied. The rate of isolation from fecal cultures that we found is much less than those reported in studies from the United States, Canada, England, and other developed countries (1, 2, 20, 21). Studies of the frequency of salmonellosis in other developing countries have shown varying results. Isolation of *Salmonella* from children with diarrhea was uncommon in Iran (1.4 per cent) (4), and Guatemala (0.5 per cent) (5); however, in South Africa and Singapore, isolation was frequent (6–8). The local factors responsible for this dichotomy have not been determined.

There are several possible explanations why the detected rate of isolation is lower in Bangladesh than in the developed countries. Surveillance may be inadequate through underreporting of illness. However, a study performed in the Matlab area has shown that most families take their children to the field clinic even when diarrhea is mild (M. Yunus, ICDDR,B, personal communication, 1980). Of the etiologic agents associated with 932 episodes of diarrheal illnesses not requiring hospitalization in 197 children from the Matlab area, *Salmonella* was isolated only once (0.1 per cent isolation rate) (R. E. Black, University of

Maryland School of Medicine, personal communication, 1980). *Salmonella* was not isolated from the feces of 850 healthy Bangladeshi adults (22). Thus, *Salmonella* was not a common cause of gastrointestinal illness or asymptomatic infection. In contrast, in Togo (9) and in South Africa (6, 7), frequent isolation of *Salmonella* from both patients with diarrhea and from healthy individuals has been reported.

Poor culture technique is a second possible explanation, yet isolating and identifying *Salmonella* is not complicated, and the techniques used at the ICDDR,B conform to established standards (15). The large proportion of cultures obtained from rectal swabs rather than from stool specimens may minimally bias the data against isolation of *Salmonella* (23). However, the test of the ability of the laboratory to isolate *Salmonella* from clinical specimens showed that the techniques in use detected salmonellae in stools at levels several logs lower than those during acute infections (24).

A third explanation for the low incidence of salmonellosis in this study is that the reservoir for *Salmonella* may be limited. In developed countries, culture surveys of poultry, swine, and cattle show a high prevalence of *Salmonella* infection (25–27), especially when animals are stressed or crowded (28, 29). Isolation of *Salmonella* from calves from English farms increased from 0.6 per cent to 36 per cent after they were held and fed together for two to five days (30). In Bangladesh, animal husbandry is much less intensive. Most animals, especially fowl, are raised and then consumed directly by the rural family. A culture survey in Dacca slaughterhouses of 282 specimens from cattle and 346 specimens from poultry showed that *Salmonella* was isolated from only 5 per cent from each. Among farm birds, the isolation rate was 1.6 per cent (M. I. Huq, unpublished data). These percentages are significantly lower than those in comparable populations of domestic animals in developed countries (25–30). In this predominantly Moslem country, there are few swine, another important reservoir in developed countries.

Another possible explanation for the low isolation rate is that the vehicles of transmission that are important in the developed countries may not be present to the same extent in Bangladesh. In the United States, salmonellosis is regarded primarily as a foodborne disease in which processed or manufactured foods of animal origin have often been implicated as vehicles, especially in outbreak situations (1). In contrast, most food consumed in Bangladesh is prepared at home and eaten immediately. The daily amount of animal protein consumed is less than 8 g per person, about one tenth the amount eaten by North Americans (31). This difference in diet may also contribute to the lower rate observed.

Although *Salmonella* infection was uncommon, we observed peaks both in the absolute number and in the rate of isolation of *Salmonella* during the months of May through August, the hottest and wettest months of the year. These peaks correspond to the seasonal distribution seen in many parts of the world (32, 33).

In the United States, half of all reported *Salmonella* cases are in children under the age of 10 years, and infants less than one year have the highest age-specific isolation rate (34). The median age of patients with salmonellosis in Matlab was not much different; teenagers and adults in Matlab were infected as frequently as children, or at least came to the hospital as frequently.

Salmonella strains isolated from some patients in developed countries have previously been shown to elaborate an enterotoxin (35, 36). Although watery diarrhea was a frequent symptom of those

with salmonellosis in this study, enterotoxins were not detected in any of the isolates. Except for two isolates (4 per cent) resistant to two antimicrobial agents each, the isolates were susceptible to all the antimicrobials tested. In Bangladesh, most farm animals graze or are fed grains produced on the farm; animal feed is rarely used and does not contain antibiotics. Although we believe that antibiotic use by humans in Bangladesh is quite limited, this has not been carefully studied. Tetracycline, because of its role in the treatment of cholera and its low cost, is the one antibiotic that may have a wider distribution (37). In comparison, in the United States in 1975, 20.8 per cent of a sample of *Salmonella* isolates were resistant to two or more antimicrobial agents, and most of these were resistant to three or more (38).

The serotypes of the *Salmonella* isolates from the two areas in Bangladesh were appreciably different from the serotypes isolated in India (39). In other parts of the world as well, there is wide variability in serotypes even within small areas, probably representing the multiple sources for *Salmonella* at any location. The dissimilarity of serotypes in Matlab and Dacca during the peaks of illness in 1977 further supports this observation.

The patients we studied probably do not represent the full spectrum of salmonellosis in Bangladesh but rather a subset with the more severe manifestations. The clinical features of salmonellosis were not much different from those we observed in other diarrheal illnesses, with the exception of cholera; however, in view of the small number of patients studied, these findings should be interpreted cautiously. Illness ranged from watery diarrhea to dysentery. In the absence of distinctive clinical features, diagnosis of *Salmonella* infection should be based on isolation from the stools. The socioeconomic backgrounds of the patients infected with *Salmonella* and those not infected were similar; however, the small number of patients and controls studied may not have been sufficient to identify differences. Further investigation is needed to determine those factors that predispose to *Salmonella* infection in Bangladesh, and those factors that protect people from salmonellosis in an area where illnesses due to other intestinal pathogens are hyperendemic.

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