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Cryptosporidium Infection and Diarrhea in Rural and Urban Areas of Jiangsu, People's Republic of China

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Screening of infants and children under age 15 years for *Cryptosporidium* oocysts in their stools was carried out in the suburb of Xuzhou City and six rural areas of Jiangsu Province. The infection rate varied from 0.7 to 5.06%. Of the total of 5,089 children examined, 89 (1.75%) were oocyst positive. The incidence was evidently higher in the group of children under age 4 years than it was in children from 4 to 15 years ($P < 0.01$). Routine blood examination and immunoassay performed on blood samples from some of the infected children indicated that more than half of them had anemia and lower cellular immunity. Diarrhea was the main symptom of cryptosporidiosis. It was intermittent or persistent and was present in 57 of the 89 children positive for *Cryptosporidium* oocysts, while the other 32 children were asymptomatic carriers. Examination of stool specimens of adult members and domestic animals of about half of the infected families showed that two mothers, one pig, and one dog were positive for *Cryptosporidium* oocysts.

Since the first case of human cryptosporidiosis was reported by Nime et al. (12) in 1976, the number of reported cases has been increasing throughout the world. Now *Cryptosporidium* spp. are recognized as worldwide pathogens of diarrheal diseases (7). However, the illness was not identified in the People's Republic of China until 1987, when Han et al. (4) reported its presence in Najing. After that, many cases of *Cryptosporidium* infection were reported from seven to eight coastal and inland provinces and the Beijing District (5, 10, 13, 14, 16), but all of the cases were detected among diarrheal outpatients in hospitals, and most of them were children. The 14 cases we detected early in July 1988 were also children who were brought to our outpatient clinic for diarrhea (OPD) for the treatment of diarrhea (2), and 3 of them were from the same village near Xuzhou. Impressed by the clinical information, we became aware that *Cryptosporidium* spp. are not the least common cause of diarrhea in children in Xuzhou, particularly when the diarrhea is refractory and protracted. In order to understand the epidemiology of *Cryptosporidium* infection in Jiangsu Province, a general survey among children under age 15 years was carried out from 1989 to 1990 by looking for *Cryptosporidium* oocysts (CSOs) in stools. The results of that survey are presented in this report.

MATERIALS AND METHODS

Selection of subjects and collection of fecal samples. The survey was carried out in one suburban village of Xuzhou City and six production brigades (areas) randomly selected from two northern, two southern, and two central counties of Jiangsu Province. The number of villages investigated totaled 34, with the number of villages investigated in any one brigade varying from 1 to 10. Single fecal samples were collected from all children under age 15 years to look for CSOs. Once a child was found to be positive, the adult members of his or her family and the domestic animals that they owned were screened as well.

Stool examination. The human stool sample was examined fresh for gross appearance and for routine ova and parasites and blood cells by emulsifying it with saline on a slide. We looked for CSOs under oil immersion on direct stool smears, which were air dried, fixed with methanol, and stained by a modified acid-fast method (6). Most examinations were done immediately after sample collection, but about a fourth of them were put off overnight. Such a delay does not affect the CSO results, because a comparative study on a dozen of the samples showed that the results were the same whether the examination was done on the same day or 3 days later. Some of the CSO-positive stool samples were cultured to detect pathogenic bacteria.

Because the animal stools contained much fine debris which would make the direct smear look cloudy and cover the expected tiny CSOs, after the smears were concentrated, they were prepared by a simplified sedimentation method (15). The fecal samples from domestic animals or poultry were first suspended and preserved in 10% formalin at 4°C. The sample was later emulsified with normal saline that was added and filtered through a copper wire sieve (4,724 holes per m^2). The filtrate was centrifuged at 500 × *g* for 15 min. The sediment was taken to make the smear, which was stained and examined for CSOs as described above for human specimens.

Clinical data and supplementary blood examinations. The CSO-positive children were interviewed individually to obtain a medical history, perform a physical checkup, and provide medical treatment for *Cryptosporidium* infection. Blood samples were also taken from some of the CSO-positive patients for routine blood examination; determination of immunoglobulin G (IgG), IgA, and IgM (single radial diffusion in agar); and lymphocyte transformation test (phytohemagglutinin method).

RESULTS

***Cryptosporidium* infections in humans.** Stool examinations showed that 89 of the 5,089 children were CSO positive. The incidence varied from 0.7 to 5.06% in different geographical areas, with an average of 1.75% (Table 1). The age distribution revealed that infants and young children, under age 4

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TABLE 1. Frequency of detection of CSOs in feces of children of Jiangsu Province

Location (brigade [no. of villages]), county	Total no. (no. M/no. F) studied[a]	CSO-positive children	
		Total no. (no. M/ no. F)	%
Suburb of city	333 (167/166)	5 (3/2)	1.50
Wuzhong (1), Xuzhou			
Northern Jiangsu			
Malin (3), Tongshan	811 (453/358)	41 (25/16)	5.06
Huxi (2), Donghai	718 (399/319)	5 (3/2)	0.70
Central Jiangsu			
Wugang (4), Lianshui	662 (354/308)	13 (7/6)	1.96
Chuanqing (7), Gaoyou	1,046 (587/459)	8 (8/0)	0.76
Southern Jiangsu			
Oingfeng (10), Lishui	684 (386/298)	7 (4/3)	1.02
Yetang (7), Changshu	835 (441/394)	10 (5/5)	1.20
Total	5,089 (2787/2302)	89 (55/34)	1.75

[a] M, male; F, female.

years, accounted for 64% (57 of 89) of CSO-positive patients (Table 2). The incidence in the group under 4 years old (57 of 1,793 [3.14%]) was significantly higher than that in the 4- to 15-year-old group (32 of 3,296 [0.97%]) ($\chi^2 = 32.92$; $P < 0.01$). The difference in incidences between the sexes was not remarkable; the incidence in boys was 55 of 2,878, and that in girls was 34 of 2,211 ($\chi^2 = 0.02$; $P > 0.05$). About three-fourths of the cases (67 of 89) were solely infected with *Cryptosporidium* spp., and the rest were concomitantly infected with *Ascaris* and/or *Giardia* spp. Examinations of adult members of families with infected children showed that two mothers had CSOs in their stools. The 359 oocysts observed in 60 samples were round, typically with four encysted spores, and were 4.37 ± 0.09 μm in diameter.

Clinical manifestations. Among the 89 infected children, 32 were asymptomatic *Cryptosporidium* carriers; the other 57 had diarrhea or had a history of diarrhea in the previous 3 months. Seven infants had suffered from diarrhea soon after birth. The most protracted diarrhea lasted for 14 months, but in most cases the diarrhea was intermittent. The children had bowel movements 4 to 5 times to more than 10 times a day. The stools were mucoid or watery and yellowish to light brown. Some children with diarrhea had other symptoms at the same time (Table 3).

TABLE 2. Incidence and age distribution of subjects infected with *Cryptosporidium* spp.

Age (yr)	Total no. (no. M/no. F) studied[a]	Oocyst-positive subjects	
		Total no. (no. M/no. F)	%
Total	5,089 (2,787/2,302)	89 (55/34)	1.75
0–1	322 (177/145)	9 (6/3)	2.30
1–2	500 (277/223)	17 (9/8)	3.4
2–3	496 (278/218)	18 (11/7)	3.63
3–4	475 (251/224)	13 (10/3)	2.74
4–6	1,075 (597/478)	15 (10/5)	1.40
6–9	940 (509/431)	8 (5/3)	0.85
9–12	891 (478/413)	9 (4/5)	1.01
12–15	390 (220/170)	0	0.00

[a] M, male; F, female.

TABLE 3. Symptomatology in 89 cases of *Cryptosporidium* infection

Symptoms	No. (%) of cases
Diarrhea	57 (64.0)
Watery stool	23 (25.8)
Mucoid stool	34 (38.2)
Nausea	10 (11.2)
Vomiting	10 (11.2)
Abdominal pain	15 (16.9)
Anorexia	20 (22.5)
Fever	8 (9.0)
Cough	2 (2.2)
No symptom	32 (36.0)

Supplementary blood and immunoassay studies. Blood and immune status were studied at two villages for 30 children with diarrhea caused by *Cryptosporidium* spp., including 21 young children (ages, <4 years), 5 preschool children (ages, 4 to 7 years), and 4 school-age children (ages, 7 to 12 years). Routine blood examination revealed a leukocyte count of 4.4 × 10^9 to 14.8 × 10^9/liter (normal range, 5 × 10^9 to 10 × 10^9/liter), the differential leukocyte count was normal, hemoglobin was under 110 g/liter in 15 cases (normal range, 110 to 150 g/liter), and the erythrocyte count was under 3.5 × 10^{12}/liter in 13 cases (normal range, 3.5 × 10^{12} to 5.0 × 10^{12}/liter), suggesting that half of the children were anemic. Determination of immunoglobulins in serum revealed an IgG level of 6.5 to 18.8 g/liter, an IgA level of 0.55 to 2.5 g/liter, and an IgM level of 0.31 to 1.88 g/liter. The individual readings were compared with the corresponding normal values for infants and children. Values for the majority of the children were within the normal range, and only a few were slightly above normal. The lymphocyte transformation test was reported to be under 0.5 (normal range, 0.5 to 0.7) for 18 cases, the lowest being 0.31, suggesting that most children with diarrhea caused by *Cryptosporidium* spp. had low cellular immunity.

Animal stool examination for CSOs. A total of 58 stool samples were collected from the animals of 29 infected families (20 pigs, 4 dogs, 5 sheep, 25 chickens, 2 ducks, and 1 goose). CSOs were found only in the smears of stool samples from one pig and one dog. The CSOs had about the same morphology and size as the human CSOs.

DISCUSSION

Cryptosporidiosis, a zoonosis characterized by diarrhea, has a worldwide distribution. Its incidence among diarrheal patients varies from 0.6 to 13% in different parts of the world (7). In the People's Republic of China, the reported number of *Cryptosporidium* diarrhea cases has been growing. They were all discovered among outpatients of hospitals in eight coastal and inland provinces and the Bejing District; these areas represent the densely to moderately populated parts of China (5, 13, 14, 16). According to our data, the incidence of *Cryptosporidium* infections in diarrheal children examined at our OPD was 3.6% (38 of 1,086). Therefore, it may be justified to say that *Cryptosporidium* infection is also widely distributed in China.

Because we were impressed by the fact that most of our

patients with diarrhea caused by *Cryptosporidium* spp. were children from nearby rural areas, the present survey was initiated to evaluate the prevalence of *Cryptosporidium* infection in children under age 15 years throughout the province. As a result, the overall incidence was found to be 1.75% (from 0.7 to 5.06%). Since the single direct smear method was used in our study, the frequency is likely to be lower and underestimates the actual prevalence. In addition, this incidence rate should not be compared with that of *Cryptosporidium* infections in outpatient groups, because the denominators of the rates are of different categories; one is the general population and the other is patients with diarrhea.

The age distribution of patients with *Cryptosporidium* infections indicates that infants and young children are much more vulnerable than older children are. Lally et al. (9) reported that 10 of 3,392 fecal samples that they surveyed were CSO positive. Among the 10 CSO-positive patients, 1 (1 of 2,581) was over age 12 years and 9 (9 of 818) were under age 12 years and were, in fact, under 4 years old. Bogaerts et al. (1) examined 293 diarrheal patients and found that 3 (3 of 100) adults and 20 (20 of 193) children were CSO positive. The average age of the 20 children was 13.3 months. Data from our outpatient group showed that the positive rate was 3.97% (32 of 806) for children under age 4 years with diarrhea and 2.4% (6 of 250) for those ages 4 to 15 years. The present survey showed that the frequency was 3.14% (57 of 1,793) for children under age 4 years and 0.93% (32 of 3,296) for those ages 4 to 15 years. The difference in frequency between age groups under and over age 4 years was very significant ($P < 0.01$ in both the outpatient group and the survey groups).

Although it is definite that the majority of *Cryptosporidium* infections are in infants and young children, the explanations for it remain hypothetical. Mølbak et al. (11) suggested that their immune functions are low so that a low-dose infection may result in cryptosporidiosis and that repeated low-dose infections may induce an immunity against *Cryptosporidium* spp., which protects the older children. In the present study, the low values of the lymphocyte transformation test suggested that the weak, immature immune systems of infants and young children are relevant to their high levels of susceptibility to cryptosporidiosis. However, additional adequately controlled studies are needed to solve the problem.

Cryptosporidiosis is well known as a zoonosis involving humans as well as animals. In our survey, oocysts were found in the smears of stool samples from a pig and a dog, and the morphologies and sizes of the oocysts were similar to those of oocysts from humans. This finding suggests that the animal and human CSOs may be of the same species. Although cryptosporidiosis can be transmitted through the animal-to-human and person-to-person routes (7, 8), our data cannot verify these routes of transmission.

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