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• BASIC RESEARCH •

Epidemiological survey of cryptosporidiosis in Anhui Province China

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Abstract

AIM: **To provide scientific evidence for prevention and controlling of cryptosporidiosis, the infection of *Cryptosporidium parvum* and its epidemiological characteristics were studied in some areas of Anhui Province.**

METHODS: **The oocyst of Cryptosporidium parvum in 5421 fresh stool samples from eleven areas of Anhui Province was tested by auramine-phenol stain and improved anti-acid stain respectively. The specific antibody of IgG, IgM and T subsets of 41 patients with positive Cryptosporidium parvum in stools were detected by ELISA and biotin-streptavidin (BSA) respectively.**

RESULTS: **The total infective rate of Cryptosporidium parvum was 1.33% (74/5421). Among them, the positive rates of oocyst in the areas of Huaibei (1.82%) and Fuyang (1.80%) were higher. The positive rates of oocyst in stools of infants, pupils, middle school students, college students, adults, patients with diarrhea, and those with immunodeficiency were 3.15%(28/889), 0.82% (9/1098), 0.82%(9/1092), 0.83%(8/969), 0.85% (9/1095), 2.88%(8/278) and 8.33%(3/36)% respectively. The positive rates of oocyst in infants and the patients with diarrhea and immunodeficiency were significantly higher than those in controls ($P<0.01$). The positive rate of oocyst in males was similar to that in females ($P>0.05$). The positive rate of oocyst in urban areas (1.13%) was significantly lower than those in rural areas (1.72%, $P<0.01$). The positive rates of specific IgG, IgM and IgG+IgM in sera of the patients with positive oocyst in stool were 63.4% (26/41), 17.1% (7/41), 19.5% (8/41) respectively. The number fractions of T subsets of CD_3^+, CD_4^+, CD_8^+ and CD_4^+/CD_8^+ of the patients were 0.66±0.07, 0.44±0.06, 0.28±0.04 and 1.58±0.32 respectively. The difference between the patients and the controls was significant ($P<0.05$). The main manifestations of the patients were subclinical infection, in forms of slight abdominal pain, mild diarrhea, and loose stool.**

CONCLUSION: **There are two infection peaks in infection of Cryptosporidium parvum and its infection can be found more often in infants, patients with diarrhea or immunodeficiency, and in rural areas. Subclinical infection is the main manifestation and might be easily misdiagnosed. When the therapeutic effectiveness is low for diarrhea, the infection of Cryptosporidium parvum should be considered, concerning their age and immune function.**

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INTRODUCTION

Cryptosporidiosis is a kind of zoonoses whose clinical manifestation is diarrhea caused by Cryptosporidium parvum[1-13]. Since the first report of the disease covered by Nime *et el*[14] in 1976, more and more studies have been reported. After the first report of the disease in 1978 covered by Hanfan *et al.* in Nanjing, many reports of the disease have been published from more than ten provinces[15-24]. In order to explore the infection, epidemiological characteristics and clinical manifestations, the investigation of the disease was taken cosmically in eleven areas of Anhui Province.

MATERIALS AND METHODS

Materials

A total of 5421 samples of stools were collected from eleven areas of Anhui Province. Among them, the number of infants, pupils, middle school students, college students, adults patients with diarrhea and immunodeficiency were 889, 1098, 1092, 969, 1373, 278 and 36 respectively. The patients with obstinate diarrhea, and immunodeficiency were the major target. The number of males and females was 3474 and 1947 respectively. The median age was 24.5 years (ranging from 4 to 63 years).

Methods

The different histories of present illness, anamnesis, health habit and healthy state of environment were taken.

Feces examination After fresh stools were collected by disposable boxes. The oocyst of Cryptosporidium parvum was tested by auramine-phenol stain and improved anti-acid stain respectively. The smears of stool were made on the surface of sheet glass (2cm^2). After these smears were left dry naturally and fixed with methanol, the auramine-phenol stain and improved anti-acid stain were made, and the examination under microscope was taken respectively. The oocyst of Cryptosporidium parvum with rose bengal was positive, and the other nonspecific granules were blue-black.

Biopsy examination The examination of colon biopsy was tested by sigmoidoscope for six patients with obstinate diarrhea, or immunodeficiency.

Serological examination The antigen of oocyst coming from guinea pigs was coated on the surface of polystyrene wells, and the specific antibodies of IgG, IgM were detected by ELISA.

Examination of T subsets The T subsets (CD_3, CD_4, CD_8) were detected by biotin-streptavidin (BSA) in 36 patients with immunodeficiency.

Statistical analysis was made by using Student's *t* test.

RESULTS

The results of oocyst of Cryptosporidium parvum in stools collected from eleven areas of Anhui Province are shown in Tables 1-4. The results of specific antibodies and T subsets are shown in Tables 5-6. The common clinical symptoms of the disease are shown in Table 7.

Table 1 The distribution of infection of Cryptosporidium parvum in Anhui Province (*n*, %)

Area	*n*	Stain of auramine-phenol		Stain of auramine-phenol and modified acid-fast	
		Positive number	Positive rate	Positive number	Positive rate
Hefei	500	4	0.80	6	1.20
Bengbu	349	2	0.57	4	1.15
Huainan	939	10	1.06	13	1.38
Lu'an	447	4	0.89	6	1.34
Wuhu	464	4	0.86	6	1.29
Huaibei	440	5	1.14	8	1.82
Huangshan	500	5	1.00	7	1.40
Fuyang	500	6	1.20	9	1.80
Chuzhou	423	4	0.95	6	1.42
Anqing	413	3	0.73	5	1.21
Suzhou	446	5	1.12	7	1.57
Total number	5421	53	0.98	74	1.33[a]

[a]$P<0.05$, $\chi^2=3.8864$.

Table 2 The distribution of infection of Cryptosporidium parvum in different groups (*n*, %)

Group	*n*	Stain of auramine-phenol		Stain of auramine-phenol and modified acid-fast	
		Positive number	Positive rate	Positive number	Positive rate
Infant	889	21	2.36[b]	28	3.15[d]
Pupil	1098	7	0.64	9	0.82
Middle school student	1092	7	0.64	9	0.82
College student	969	5	0.52	8	0.83
Adult	1059	6	0.57	9	0.85
Patients with diarrhea	278	5	1.80[a]	8	2.88[d]
Patients with immunodeficency	36	2	5.56[b]	3	8.33[d]
Total number	5421	53	0.98	74	1.33

[a]$P<0.05$, [b]$P<0.01$, [d]$P<0.01$, *vs* college students.

Table 3 The distribution of infection of Cryptosporidium parvum in different sexes (*n*, %)

Group	*n*	Stain of auramine-phenol		Stain of auramine-phenol and modified acid-fast	
		Positive number	Positive rate	Positive number	Positive rate
Males	3474	37	1.07[d]	59	1.70[d]
Females	1947	17	0.87	30	1.54
Total number	5421	54	0.99	89	1.64

[d]$P>0.05$, *vs* female.

Table 4 The distribution of infection of Cryptosporidium parvum in urban and rural areas (*n*, %)

Group	*n*	Stain of auramine-phenol		Stain of auramine-phenol and modified acid-fast	
		Positive number	Positive rate	Positive number	Positive rate
Urban areas	3276	26	0.79[a]	36	1.13[a]
Rural areas	2145	27	1.26	38	1.72
Total number	5421	53	0.98	74	1.33

[a]$P<0.05$, *vs* Rural.

Table 5 The detective results of specific antibody against *Cryptosporidium parvum* (*n*, %)

Oocyst	*n*	IgG		IgM		IgG+IgM	
		Positive number	Positive rate	Positive number	Positive rate	Positive number	Positive rate
Positive[b]	41	26	63.41	7	17.07	8	19.51
Negative	20	0	0.00	0	0.00	0	0.00

[b]$P<0.01$, *vs* Negative, $\chi^2=25.0945$.

Table 6 The results of T subsets of patients with positive of Cryptosporidium parvum in stool (*n*, $\bar{x}\pm s$, %)

cryptosporidium parvum	*n*	CD_3^+	CD_4^+	CD_8^+	CD_4^+/CD_8^+
Positive	41	65.83±6.55[a]	43.55±6.10[a]	28.43±4.32	1.58±0.32[b]
Negative	20	55.87±7.23	39.26±6.43	30.04±5.67	1.36±0.41

[a]$P<0.05$, [b]$P<0.05$, *vs* Negative.

Table 7 Clinical symptoms after being infected by Cryptosporidium parvum (*n*, %)

Group	*n*	percentage
Without symptom	62	83.78
Symptom	12	16.22
General symptom	2	2.70
Upper digestive tract symptom	1	1.35
Lower digestive tract symptom	2	2.70
Upper and lower digestive tract symptom	3	4.05
General and upper digestive tract symptom	1	1.35
General and lower digestive tract symptom	2	2.70
General and upper, lower digestive tract symptom	1	1.35
Total number	74	100.00

General symptom:acratia, fever;upper digestive tract symptom:anorexia, nausea, vomiting;Lower digestive tract symptom:abdominal distension, abdominal pain, loose stool, watery stool

Examination of living tissue The examination of biopsy was tested by sigmoidoscope in six adults and old patients with obstinate diarrhea, or immunodeficiency. The results showed that there were many oocysts on the surface of intestinal mucosa, which had villus degeneration and mononuclear leukocyte infiltrations.

DISCUSSION

Cryptosporidium parvum is recognized as an important protozoon, whose life cycle is simple with nonspecific host. Large-scale surveys of selected animals suggest that Cryptosporidium parvum is more often found in farm cattle, sheep, dogs and cats. The disease can be transmitted in animals and people mutually. Water polluted with Cryptosporidium parvum is regarded as a source of infection by some experts[24-35]. Patients with immunodeficiency (AIDS) can easily be infected through respiratory tract. During the gastroenteritis of fulminant epidemic, the positive rate of Cryptosporidium parvum was 39% in 13000 patients with gastroenteritis[14].

The pathogenicity of Cryptosporidium parvum hasn't been, for a long time, taken into serious consideration. Since the report of severe diarrhea caused by Cryptosporidium parvum breaking out in Turkeys in 1955 (Stavin), and in 1976 (Nime)[14], the infection of the disease has been reported in many countries. The different positive rates of Cryptosporidium parvum are 1-2% in Europe, 0.6-4.3% in North America, and 3-4% or even 10.2% in Asia, Australia, Africa, and Central and South America.

The pathogenic mechanism of Cryptosporidiosis hasn't been clarified. The report of Hanfan (1990)[15,16] showed that after the infective oocyst invaded the intestine, its sporozoites intruded epithelium mucosae villus and its larva could reproduce in vacuole. With the development of the disease, the epithelium mucosae villus would collapse, light or medium inflammatory reaction with mononuclear leukocytes and watery stool could appear. Decreasing the

activity of lactase caused by infection of Cryptosporidium parvum was an important reason for losing lactose and diarrhea[36-46].

Since the first patient with the disease was diagnosed in 1987 in our country, many cases of the disease have been reported, especially in Jiangsu, Fujian, Hunan, Shandong Provinces and Inner Mongolia. The total detective rate of the disease was 1.36-13.3%. It was more often found in infants and children[47-55]. Our data of investigation suggest that the infection of Cryptosporidium parvum has existed in Anhui Province, and its detective rate was low (1.33%, 74/5421)[15,16]. The infective rate of Cryptosporidium parvum in males and females was 1.41% and 1.28% respectively. There was no significant difference between two sexes (P>0.05). Although stain of auramine-phenol is one of the good methods for the detection of oocyst, the specificity of stain can be interfered by impurity in stool. The stain of auramine-phenol and modified acid-fast can overcome false positive reaction and false negative reaction of oocyst so that the detective rate of oocyst can be increased (P<0.05). The infectious rates of Cryptosporidium parvum were higher in infants and patients with obstinate diarrhea or immunodeficiency than those in middle school students and college students (P<0.01). The possible reason was immunodeficiency, lower positive rate of CD_3^+, CD_4^+ and CD_4^+/CD_8^+, so that the patients had not enough immune reaction to Cryptosporidium parvum. The similar results of the isolation rate had been observed in our investigation, which was more often found in infants and children with diarrhea. The possible reason was the immune organs of infants and children hadn't matured. After Cryptosporidium parvum invaded the intestine, the structure of pithelium mucosae villus was demolished and few antibodies were produced. The extent of the disease for adults was not only associated with the level of infection of Cryptosporidium parvum but also associated with the immunity. It was more often found in parasite states and self-limited diarrhea for normal population. It was more often found in severe infection and continuous diarrhea for immunodeficiency. Scavenger worm was associated with the level of Th and ADCC of the patients. The production of restrain factor and the decrease of T cells and T subsets were caused by the common antigen of different enteric bactilli and infected epithelial cells of colon. For the patients with low or no treatment effect of antibiotic, taking into consideration their living environment and individual living habits, the possible infection of Cryptosporidium parvum should be considered. In the study of Pan *et al*[56-62], for thirteen patients with ulcerative *coli*tis and ten patients with clone disease, the function of T cells and restrain index number were all deficient in the patients with inflammation intestinal tract. The infective rate of Cryptosporidium parvum was higher in rural areas than that in urban areas (P<0.05). The possible reasons were poor living conditions, lack of necessary general health knowledge and health habits in the rural areas. Food and drinking water polluted by oocyst was the possible cause of the infection.

The main antigen of Cryptosporidium parvum was cyst wall antigen and sporozoite antigen. Most scholars considered that cellular immunity was important and the immune mechanism of cryptosporidiosis hadn't been clarified. Moon's study showed that IgG, IgM against Cryptosporidium parvum couldn't repress the infection, so that the immunity of cryptosporidiosis was dependent on cellular immunity. However, other scholars, for example Chrisp and Riggs, thought that the specific antibody could easily be made after adult and young mice were vaccinated by oocyst. The detective results of specific IgA, IgM, IgG in serum, stool and duodenal juice and cellular immune function prompted that the immunity of cryptosporidiosis was dependent on ADCC. The results of our study showed that type of antibody most frequently found was IgG, with IgM, and IgG+IgM following it. For IgM as target of early infection was not necessarily a verified index, if IgG or IgM in serum was positive, possible infection of Cryptosporidium parvum should be considered. The positive effects of circle antibody haven't been completely clarified according to the previous results that the circle antibody hadn't protective function[15-17]. It is possible that the effect of antibody in serum against Cryptosporidium parvum in intestinal pithelium mucosae villus is ineffective.

The expressive levels of CD_3^+, CD_4^+ and CD_4^+/CD_8^+ were lower in positive rates of oocyst in stool than those in negative rates of oocyst in stool (P<0.05). The result showed that the cellular immunity played a key role against the infection of Cryptosporidium parvum. When the levels of CD_3^+, CD_4^+ were low, the activity of T cells and its cellular factor were inadequate, and the infection of Cryptosporidium parvum would persist. However, the result of general level of CD_8^+ in the patients with positive rates of oocyst indicated that the activity and number of CTL hadn't significantly increased, and severe tissue injuries, generally speaking, wouldn't take place in the patients.

Most patients neglected diagnosis and treatment when they had no or light symptoms. Most people with normal immune functions suffering from self-limited diarrhea often had symptoms of acute watery stool (5-10times/d), nausea, vomiting, headache etc, and their course of disease was less than one month. The results of our study showed that about 83.78% infected persons had no obvious symptoms, the possible reason for it was associated with the infective level and the ability of immune response. The symptoms of the patients were easily confused with general gastroenteritis. If the treatment of antibiotic failed, the infection of this disease should be considered, eliminating some associated diseases.

As a conclusion, there were two infection peaks in the infection of cryptosporidium parvum, and the infection of Cryptosporidium parvum has existed in Anhui Province, and was more often found in infants, children and some patients with diarrhea or immunodeficiency. The effect of specific IgM, IgG in sera of the patients against Cryptosporidium parvum in intestine was much inferior. If the treatment of antibiotic failed, the infection of this disease should be considered, considering age and immune function of the patients, if some associated diseases are eliminated. In order to avoid the persistent and chronic state of the illness, antiscolic treatment must be taken earlier for the subclinical infective patients with confirmed diagnosis.

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