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An epidemiological survey of *Cryptosporidium parvum* infection in randomly selected inhabitants of Seoul and Chollanam-do

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Abstract: An epidemiological survey was performed to know the status of *Cryptosporidium* sp. infection among the people in Seoul and Chollanam-do in 1992. One village of Chollanam-do (Hwasun-gun) which showed the highest oocyst positive rate was re-surveyed in 1995 for human infection and for cattle also. The subjected areas consisted of 8 urban villages (= dongs) of Seoul and 4 urban (= dongs) and 7 rural (= myons) villages of Chollanam-do. A total of 3,146 fecal samples was collected randomly, and smears were made from formalin-ether sediments. They were examined for *Cryptosporidium* oocysts by modified acid fast staining. The overall oocyst positive rate was 7.9% (248/3,146), but the rate was remarkably different between Seoul and Chollanam-do, 0.5% (4/853) and 10.6% (244/2,293), respectively. The average size of oocysts was 4.8 ± 0.5 by 4.2 ± 0.5 μm, compatible with *C. parvum*. In Chollanam-do, rural villages showed significantly higher rate (14.0%) than urban villages (3.7%). Especially the people in Iyang-myon, Hwasun-gun, a typical rural village, revealed a very high rate of 40.0% (74/185). Adults aged 51-70 years revealed the highest positive rate among all age groups. At the re-survey of the same village of Hwasun-gun in 1995, 44 (35.2%) of 125 villagers and 14 (93.3%) of 15 cattle examined were positive for *C. parvum* oocysts. The results suggest that *C. parvum* is highly prevalent in rural areas of Chollanam-do, and an important source or mode of infection seems to be contaminated water or contact with the feces of infected cattle.

Key words: *Cryptosporidium parvum*, infection status, people, cattle, Seoul, Chollanam-do, Iyang-myon (Hwasun-gun), modified acid fast stain

INTRODUCTION

Since the discovery of the sporozoan parasite *Cryptosporidium muris* in mice by Tyzzer (1907), infections with *Cryptosporidium* spp. have been detected in a wide range of vertebrate hosts throughout the world (Fayer and Ungar, 1986; O'Donoghue, 1995). The most important species infecting humans is known to be *C. parvum* (Current and Garcia, 1991), and another possible species infecting man includes *C. baileyi* (Ditrich *et al.*, 1991).

After the first discovery of human cryptosporidiosis (Nime *et al.*, 1976; Meisel *et al.*, 1976) not so many papers have been published on human infection until 1982, but after that time this disease became widely recognized as a life-threatening infection in acquired immune deficiency syndrome (AIDS)

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patients (Fayer and Ungar, 1986). Nowadays medical interest on cryptosporidiosis is increasing remarkably. A lot of epidemiological surveys on the status of human infection has been done in various countries.

In Korea experimental studies on *Cryptosporidium* were started by Chai *et al.* (1990), who first verified the presence of *C. parvum* in laboratory mice by immunosuppression with prednisolone. Later Rhee *et al.* (1991) reported prevalence of *Cryptosporidium* spp. in laboratory mice, house rats, chickens, milk cows, and pigs as 29.6%, 13.3%, 15.0%, 19.6%, and 22.2%, respectively. Wee *et al.* (1996) found 29 (14.4%) oocyst positive calves among 201 examined.

Status of human infection with *Cryptosporidium* was first studied by Cho *et al.* (1993), who reported 22% fecal oocyst positive rate out of 230 randomly selected out-patients of Severance Hospital. Recently, Kang *et al.* (1995) reported a leukemic child with severe diarrhea who revealed various life cycle stages of *C. parvum* in the resected appendix at electron microscopy. However, the status of *Cryptosporidium* infection among healthy individuals has never been reported. The present study was undertaken to know the infection status of urban and rural villagers of Seoul and Chollanam-do with *C. parvum*.

MATERIALS AND METHODS

1. Samples and areas surveyed

We used the same fecal samples collected randomly by the Korea Association of Health for the fifth national survey on the prevalence of intestinal parasitic infections in Korea (Ministry of Health and Social Welfare & Korea Association of Health, 1992). Among 46,912 samples collected nationwidely a total of 3,146 from 8 urban villages (= dongs) of Seoul and 4 urban (= dongs) and 7 rural (= myons) villages of Chollanam-do were selected for this study.

The number of specimens from Seoul was 853, which included 163 from Hyochang-dong, 136 from Hannam 1-dong, 110 from Imun 2-dong, 56 from Changan 2-dong, 106 from Changshin 2-dong, 104 from Chungnim 4-dong, 88 from Chonnong 4-dong, and 90 from Chegi-dong.

The number of samples from 4 urban villages of Chollanam-do was 751, which consisted of 209 from Mokpo-shi (city) Sosan-dong, 138 Mokpo-shi Taesong 2-dong, 172 Yosu-shi Kwanmun-dong, and 232 Yosu-shi Kuk-dong. The total number of samples from 7 rural areas of Chollanam-do was 1,542, which consisted of 184 from Haenam-gun Kyegok-myon, 275 Naju-gun Pannam-myon, 253 Changhung-gun Changhung-up, 236 Sungju-gun Pyollyang-myon, 185 Hwasun-gun Iyang-myon, 182 Koksong-gun Okkwa-myon, and 227 Yochon-gun Sora-myon.

2. Second survey in Hwasun-gun

As Hwasun-gun Iyang-myon revealed very high oocyst positive rate in 1992, the area was re-surveyed in April, 1995. A total of 125 fecal samples were collected from the people of the same village, and examined for *C. parvum* oocysts. Searching for the source of human infection, fecal samples of 15 cattle reared in the village were also collected, and examined for the presence of *C. parvum* oocysts.

3. Methods of oocyst detection

Fecal smears were prepared by formalin-ether sedimentation, and examined after modified acid fast staining (Garcia *et al.*, 1983). The smears were observed by light microscope under high power magnification (× 1,000). Presence of *Cryptosporidium* oocysts of 4-5 μm in diameter, red color and containing sporozoites, was judged as positive.

RESULTS

1. Oocyst positive rate of the surveyed population

Out of total 3,146 samples collected from Seoul and Chollanam-do, 248 (7.9%) were positive for the oocysts of *Cryptosporidium* (Table 1). The oocyst positive rate of people in Chollanam-do was 10.6%, remarkably higher than 0.5% of people in Seoul (Table 1).

2. Species of *Cryptosporidium* based on the size of oocysts

The size of oocysts, 60 in number from randomly selected positive samples, was (4.8 ±

Table 1. Overall *C. parvum* oocyst positive rate of the surveyed population

District	No. exam.	No. positive	Positive rate (%)
Seoul	853	4	0.5
Chollanam-do	2,293	244	10.6
Total	3,146	248	7.9

Table 2. *C. parvum* oocyst positive rate by surveyed areas of Seoul

Area	No. exam.	No. positive	Positive rate (%)
Hyochang-dong	163	0	0.0
Hannam 1-dong	136	0	0.0
Imun 2-dong	110	1	0.9
Changan 2-dong	56	0	0.0
Changshin 2-dong	106	1	0.9
Chungnim-dong	104	0	0.0
Chonnong 4-dong	88	1	1.1
Chegi-dong	90	1	1.1
Total	853	4	0.5

0.5) × (4.2 ± 0.5) μm, a little small but compatible with *C. parvum* (Upton and Current, 1985).

3. Oocyst positive rate by areas surveyed

Slight but not so significant difference in the oocyst positive rate was observed among the urban villages (= dongs) surveyed in Seoul (Table 2). No people was found infected in Hyochang-dong, Hannam 1-dong, Changan 2-dong and Chungnim-dong. One case each was positive for the oocysts in Imun 2-dong, Changshin 2-dong, Chonnong 4-dong and Chegi-dong, with the positive rate of 0.9%, 0.9%, 1.1% and 1.1%, respectively (Table 2).

In Chollanam-do remarkable difference was noted in the oocyst positive rate between urban and rural villages, and by each village (Table 3). In 4 urban villages of Sosan-dong and Taesong 2-dong of Mokpo City, and Kwanmun-dong and Kuk-dong of Yosu City, the average oocyst positive rate was 3.7%, and by each village, 2.9%, 5.1%, 0.6%, and 6.0%, respectively. The positive rate was more remarkably different among the rural villages of Chollanam-do. The average oocyst positive rate in rural villages was 14.0%, but the rate ranged from 6.2% in Naju-gun Pannam-myon to 40.0% in Hwasun-gun Iyang-myon (Table 3).

4. Oocyst positive rate by age and sex of the surveyed population

The age distribution of the surveyed population was typical pattern of general population in Korea. The oocyst positive rate was 3.2% in the age group 0-10, the lowest of all age groups, but increased to 4.9-5.6% in 11-40 year groups, and 9.0% in 41-50 year group (Fig. 1). A peak positive rate was noted in the age groups 51-60 and 61-70, 13.2% and 15.9%, respectively. Later than 71 years, the positive rate decreased a little, 7.9% (Fig. 1).

Of the samples examined, 1,374 were males, 1,727 were females, and remaining 45 cases had no record on the gender. The oocyst positive rate of males was 8.4% and that of females was 7.7% (Table 4), but the difference was statistically not significant.

5. Density of oocysts in fecal smears

In most of the infected people, 3-5 oocysts were detected in each fecal smear. Less than 10% of the infected people revealed more than 10 oocysts per fecal smear.

Table 3. *C. parvum* oocyst positive rate by urban and rural villages of Chollanam-do

Area	No. exam.	No. positive	Positive rate (%)
Urban areas			
Mokpo-shi Sosan-dong	209	6	2.9
Mokpo-shi Taesong 2-dong	138	7	5.1
Yosu-shi Kwanmun-dong	172	1	0.6
Yosu-shi Kuk-dong	232	14	6.0
Subtotal	751	28	3.7
Rural areas			
Haenam-gun Kyegok-myon	184	33	17.9
Naju-gun Pannam-myon	275	17	6.2
Changhung-gun Changhung-up	253	22	8.7
Sungju-gun Pyollyang-myon	236	26	11.0
Hwasun-gun Iyang-myon	185	74	40.0
Koksong-gun Okkwa-myon	182	17	9.3
Yochon-gun Sora-myon	227	27	12.0
Subtotal	1,542	216	14.0
Total	2,293	244	10.6

Table 4. Positive rate of *C. parvum* oocysts according to sex of the people surveyed

Sex	No. exam.*	No. positive	Positive rate (%)
Male	1,374	115	8.4
Female	1,727	133	7.7

*The gender of remaining 45 cases is not known.

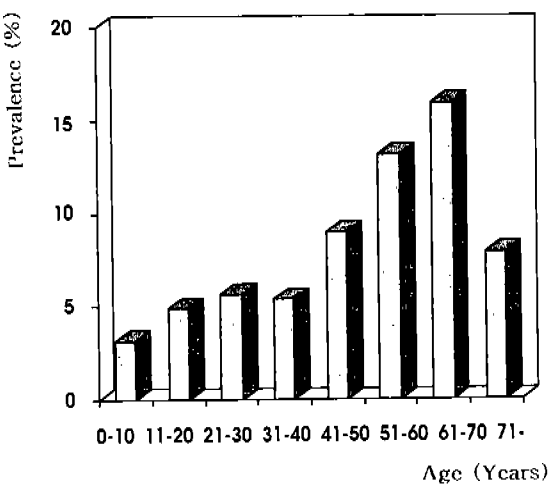

Fig. 1. Age-prevalence of *C. parvum* infection among the surveyed population in Seoul and Chollanam-do, 1992.

6. Results of the second survey in Hwasun-gun

In order to verify that the high prevalence of *C. parvum* oocysts among the people in Hwasun-gun Iyang-myon in 1992 was not an incidental finding, a second survey was performed on the same village in April, 1995. The total number of people examined was 125, and 44 (35.2%) were positive for *C. parvum* oocysts (Table 5).

The age group 0-10 revealed the lowest positive rate (11.1%), and the age group 41-50 showed the highest positive rate (55.6%). People older than 41 years showed significantly higher positive rate than people younger than 40 years of age (Table 5). Several infected villagers recalled they had episodes of diarrhea and/or constipation, or gastric cramp after ingestion of alcoholic beverages. However, many of the infected people were asymp-

Table 5. Second survey results on the prevalence of *C. parvum* by age of the people in Hwasun-gun Iyang-myon (April, 1995)

Age group	No. exam.	No. positive	Positive rate (%)
0-10	9	1	11.1
11-20	8	2	25.0
21-30	3	1	33.3
31-40	7	2	28.6
41-50	9	5	55.6
51-60	31	9	29.0
61-70	30	10	33.3
71 & over	28	14	50.0
Total	125	44	35.2

tomatic.

Searching for the source of infection, the feces of 15 cattle reared in this village were collected and examined. Fourteen (93.3%) were found positive for the oocysts of *C. parvum*. Three of them were severely infected, excreting a large number of oocysts.

DISCUSSION

On the prevalence of human cryptosporidiosis, numerous reports have been published in many countries. In North America non-outbreak-associated prevalence rates ranged from 0.6% to 4.3% by areas (Fayer and Ungar, 1986). In Europe, prevalence was most often between 1% and 2% of population with diarrhea or other gastrointestinal troubles (Fayer and Ungar, 1986). In patients with diarrhea, *Cryptosporidium* oocysts were found in 13.1% in India, and 16.5% in Haiti (Webster, 1993). Among AIDS patients 10% developed cryptosporidiosis in the US and developing countries (Webster, 1993). In contrast, prevalence rates in Asia, Australia, Africa, and Central and South America generally began at 3-4% and reached 10-20% (Fayer and Ungar, 1986).

Comparing with the above figures 0.5% oocyst positive rate of Seoul in this study was low. In contrast, it was surprising that rural villagers of Chollanam-do revealed high positive rate, 10.6% on average. Especially Hwasun-gun showed the highest rate, 40%. A second survey on the same village in 1995 showed 35.2% oocyst positive rate. These were extremely high values, strongly suggesting high endemicity of cryptosporidiosis in this area possibly associated with infected cattle or contaminated water, both of which are known to be important sources of infection with *Cryptosporidium* (Jokipii *et al.*, 1983; D'Antonio *et al.*, 1985; Fayer and Ungar, 1986). In Hwasun-gun, 14 of 15 cattle examined were positive for *C. parvum* oocysts, and three of them excreted numerous oocysts. There is a big water reservoir nearby the village, and pipe water is supplied. But the surrounding of the reservoir is not so clean, and feces of cattle or other wild animals can come into the reservoir on rainy days. As the individual hygiene of the villagers in Hwasun-gun was not so good, direct contact with the feces of cattle could also be a possible mode of infection.

In this study we regret that clinical details of infected people were not available. It is uncertain whether some of them were under immunosuppressed condition or not. Their clinical symptoms were not investigated. At the second survey in Hwasun-gun, however, several infected villagers recalled they had episodes of transient diarrhea, constipation, or gastric cramp after ingestion of alcoholic beverages. But many of them were co-infected with other intestinal parasites such as *Metagonimus* sp. (data not shown), so that it is unreliable to correlate such symptoms with *C. parvum* infection alone.

The age-prevalence pattern of *C. parvum* infection in this study was quite characteristic and different from that of other countries (Fayer and Ungar, 1986). In this study a strong tendency was observed that the older

the age group the higher the infection rate. It is contrasting to the statement that *C. parvum* infection occurs preferably in young children under 5 years old, presumably due to their immunological immaturity and their general risk of infection due to hygienic behavior (O'Donoghue, 1995).

This discrepancy is difficult to explain, since it is well known that *C. parvum* infection does not last longer than 1-2 weeks in immunocompetent individuals (O'Donoghue, 1995). A possible speculation is that elder people in the surveyed rural villages had more and repeated chance of exposure to oocysts than young children. At the second survey in Hwasungun, a similar age-prevalence was noted again, suggesting strongly that it is a consistent feature in the surveyed areas. So as to understand properly the reason for this peculiar age-prevalence pattern, immunological competence of elder people in rural areas of Korea should be evaluated in the future.

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=초록=

서울 및 전남 지역 주민의 작은와포자충 감염에 대한 역학조사

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서울 및 전남 지역 주민의 작은와포자충(*Cryptosporidium parvum*) 감염 상황을 알아보기 위하여 1992년에 표본조사를 실시하였다. 한편, 높은 오시스트 양성률을 보인 전남 화순군 이양면에 대해서는 1995년에 재조사를 시행하였다. 구체적 대상 지역은 서울의 8개 동(洞) 및 전라남도 도시지역 4개 동 및 농촌지역 7개 면으로 하였다. 총 3,146명을 무작위로 선정하여 분변을 채취하였고, 포르말린-에텔법으로 침전시킨 다음 침사로부터 도말표본을 1인 1매 제작하였다. 표본을 modified acid fast 법으로 염색한 후 작은와포자충의 오시스트 양성 여부를 조사하였다. 총 오시스트 양성률은 7.9%(248/3,146)이었으나, 서울 0.5%(4/853) 및 전라남도 10.6%(244/2,293)로서 지역별로 큰 차이를 보였다. 오시스트의 크기는 4.8 ± 0.5 × 4.2 ± 0.5 μm로 *C. parvum*에 부합되었다. 전라남도의 경우 농촌 지역은 14.0%로 도시 지역의 3.7%보다 유의하게 양성률이 높았다. 특히, 대표적인 농촌 지역의 하나인 화순군 이양면의 경우에는 40.0%(74/185)의 매우 높은 양성률을 나타내었다. 전체 조사 대상자에서 볼 때 51-70세 연령군이 모든 연령군 중 가장 높은 양성률을 보였다. 화순군 이양면에 대한 제2차 조사(1995년)에서는 주민 125명 중 44(35.2%)명이, 소 15두 중 14두(93.3%)가 분변내 오시스트 양성이었다. 이상의 결과로 전라남도 농촌 지역이 작은와포자충의 농후 유행지임을 알 수 있었고, 중요한 감염원(감염 방식)으로는 오염된 물이나 감염된 소와의 접촉일 것으로 추정되었다.

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