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Cryptosporidiosis in Poland: clinical, epidemiologic and parasitologic aspects

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Abstract. Stool samples from 692 children hospitalized with symptoms of gastroenteritis, were examined for *Cryptosporidium parvum*. The oocysts were found in 17 (2.4%) children. There was no association with age, sex or location of household. However, relatively higher rate of oocyst exclusion was found among 2-month- to 3-year-old groups (50%) and among children from villages (43%). Six children from the group of infected were additionally screened for general immunocompetence. Four of these exhibited signs of immunodepletion of IgA isotype in serum. This study has established that cryptosporidiosis occurred rather sporadically among children in the examined groups.

Further study was undertaken to provide relevant information whether wild small mammals are a zoonotic reservoir of *Cryptosporidium* sp. Samples were collected from autumn 1989 until spring 1991 in northern Poland, District Mazury Lake. Twenty percent (66 of 330) of the examined mammals were naturally infected with *Cryptosporidium* sp.: 55 of 275 *Clethrionomys glareolus*, 6 of 39 *Apodemus flavicollis*, and 5 of 16 *Sorex araneus* were positive. The histological study clearly indicates that the population of *C. glareolus* was infected with *C. parvum*. Endogenous stages were found in duodenum near the pylorus. The intensity of infection in those animals was generally low, and relatively small numbers of trophozoites and oocysts were found. This report indicates that *C. glareolus* and possibly other rodents have the potential to act as reservoirs for *C. parvum*.

Infections with coccidia of the genus *Cryptosporidium* Tyzzer, 1910 have been described in fish, reptiles, birds and mammals including man (Boch et al. 1982, Hoover et al. 1981, Tzipori 1983). Cryptosporidia are parasites of the alimentary and respiratory mucosae, invading the microvillus border of the enterocytes. Infections associated with diarrhoea and enteric lesions have been reported in a variety of animals and in humans (Meisel et al. 1976, Morin et al. 1976, Weisburger et al. 1979, Bird and Smith 1980, Tzipori 1983,). Although the importance of *Cryptosporidium* sp. as an intestinal pathogen in both immunocompetent and immunocompromised persons has become increasingly more apparent (Current et al. 1983), little information is available on the prevalence, significance and prognosis of cryptosporidiosis in Poland (Siński et al. 1988). Similarly, the zoonotic potential of wild rodents acting as reservoirs for the transmission of *Cryptosporidium* to other mammals, including man, has not been evaluated.

In this study, a report on the prevalence of cryptosporidiosis among children in Poland is presented. The study was based at the Children's Memorial Hospital in Warsaw, to which patients are referred from districts throughout Poland, and involved children hospitalized with a variety of gastrointestinal symptoms all of whom had severe diarrhoea. In addition, the prevalence of *Cryptosporidium* among the wild small-mammal populations and the characteristics of *Cryptosporidium* found in these hosts are reported.

MATERIALS AND METHODS

Screening of children for *Cryptosporidium* sp. infection

The studies were carried out in the Children's Memorial Hospital, Warszaw during a 5-year period. A total of 692 children (2 months to 17 years old) with symptoms of gastroenteritis were examined. Faecal smears were prepared directly from each patient without concentration, mostly loose or liquid specimens, air dried, fixed in methanol, and stained by the Ziehl-Neelsen technique as described by Henriksen and Pohlenz (1981). All slides were examined for *C. parvum* oocysts using 400x magnification, with confirmation under oil immersion at a magnification of 1000x. A preparation was considered negative only after screening more than 100 fields. Examinations for other parasites in specimens were carried out routinely.

The immunological status of children infected with *Cryptosporidium* sp. was assessed by measurements of total serum IgG, IgM and IgA levels, blast transformation test, E rosette test, and nitro-blue tetrazolium test by conventional techniques as requested by clinicians responsible for individual cases.

Screening of small mammals for *Cryptosporidium* sp. infection

The study was conducted in northern Poland, in the District of Mazury Lake (Field Station of the Department of Ecology, Zoological Institute, University of Warszawa, Urwitałt, near Mikołajki). Five species of live-trapped small mammals, *Clethrionomys glareolus, Apodemus flavicollis, A. agrarius, Sorex araneus,* and *S. minutus* were examined. The animals were collected four times: in autumn 1989, in spring and autumn 1990 and in spring 1991 using box-traps. Animals were euthanased using ether. At necropsy the stomach, duodenum, ileum and jejunum from all animals were removed and histological sections of these

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parts of intestine were prepared. Paraffin sections were stained with hematoxylin and eosin and examined for the presence of endogenous stages of *Cryptosporidium*. Faecal and/or colon contents smears, stained with Ziehl-Neelsen method, were examined for *Cryptosporidium* oocysts. The estimation of *C. parvum* was assessed histologically on the basis of localization of endogenous stages of parasites.

RESULTS

Cryptosporidium sp. oocysts were found in 17 (2.4%) of 692 children examined. All infected children had diarrhoea. In one particular case, a one-year-old boy, with heavy diarrhoeal symptoms and with hypogammaglobulinemia A, shed cryptosporidial oocysts for over 28 days. The screening of *Cryptosporidium* sp. by Ziehl-Neelsen staining permits the reaching of a presumptive diagnosis. The oocysts were pink to red, measured 4 to 6 µm, and were round to oval, containing four crescent-shaped sporozoites. The age and sex distribution of children with cryptosporidiosis is presented in Fig. 1. There was no correlation of prevalence of *Cryptosporidium* sp. infection with sex or age, although relatively more boys (57%) than girls (43%) were infected. In addition, higher rates of oocysts were observed among children less than 3 years old compared with older groups. The prevalence of *Cryptosporidium* sp. did not differ significantly between groups of children originating from different regions of Poland. However, relatively higher rates of oocyst production were observed among children from villages (43%) compared with groups from towns (21%) and cities (36%).

Nine of all *Cryptosporidium* positive cases were associated with other parasitic organisms: 3 with bacteria, 4 with *Blastocystis hominis*, one with *Giardia lamblia*, and one with *Ascaris lumbricoides*.

Six children of the 17 infected were additionally screened for general immunocompetence. Four of these exhibited signs of immunodepletion of IgA isotype in serum. None of the children positive for *Cryptosporidium* sp. had received any immunosuppressive chemotherapy.

The screening of Ziehl-Neelsen stained faecal smears from small mammals in the District of Mazury Lake revealed that of 330 examined animals 66 (20%) shed oocysts: 55 of 275 *C. glareolus*, 6 of 39 *A. flavicollis*, 5 of 16 *S. araneus* were positive for oocysts of *Cryptosporidium*. The prevalence of *Cryptosporidium* sp. infection in all examined host species in 1989 to 1991 is shown in Table 1. Faecal examination revealed that 20% of *C. glareolus*, 15.4% of *A. flavicollis* and 31.3% of *S. araneus* were infected with *Cryptosporidium,* however these animals were shedding oocysts in a very low concentration (mean 5 oocysts per 10 fields, x160). The infection rates for *C. glareolus* were relatively high in autumn 1989 and in spring 1990, 30.0 and 23.1%, respectively; and in autumn 1990 and spring 1991, 13.9 and 14.0%. The infection rate

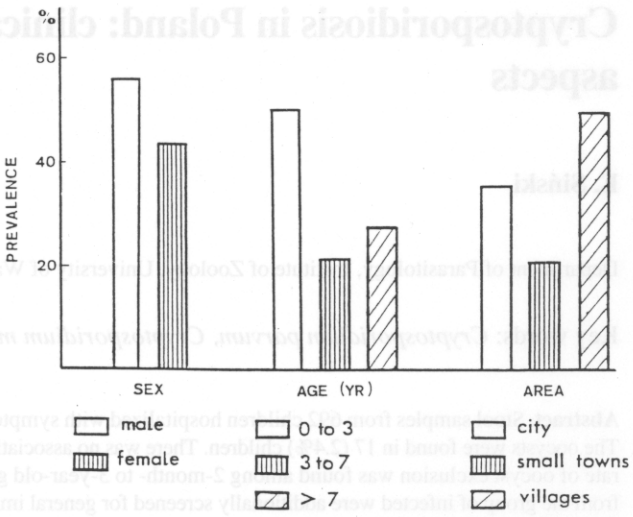

Fig. 1. Environmental specific prevalence of *Cryptosporidium parvum* in children with gastroenteritis; relation to age and sex.

was higher in *S. araneus* during the spring 1990 and in *A. flavicollis* during the spring 1991. On histological examination, the endogenous stages of *Cryptosporidium* were found in the upper part of the small intestine (duodenum and jejunum) in *C. glareolus* (Fig. 2). There was no evidence of *Cryptosporidium* infection following both coproscopic and histological examination in *A. agrarius* and *S. minutus*.

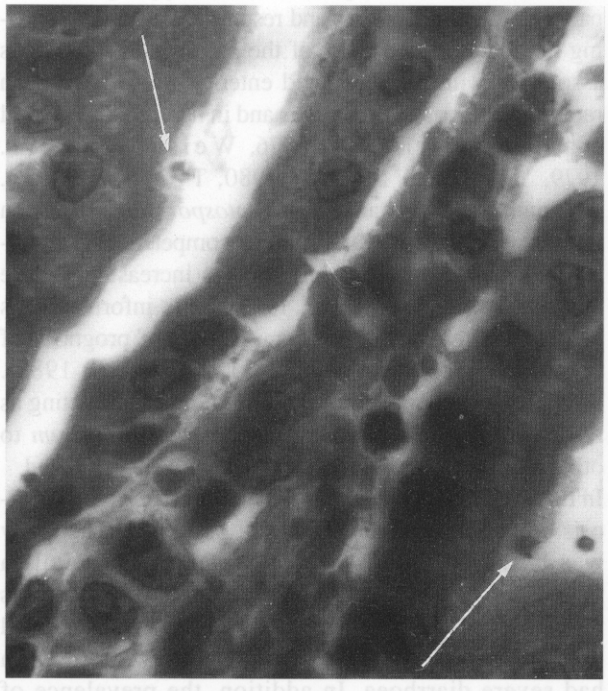

Fig. 2. Light micrograph of *Cryptosporidium parvum* in the small intestine of *Clethrionomys glareolus*. Endogenous parasite stages (arrows) at the luminal surface of the intestinal epithelium. Hematoxylin and eosin (x500).

Table 1. Mean infection rates of *Cryptosporidium* sp. in wild small mammals.

Season		*C. glareolus*			*A. flavicollis*			*S. araneus*		
		a	b	c	a	b	c	a	b	c
Autumn	1989	60	18*	30.3	14	3	21.4	8	3	37.5
Spring	1990	78	18	23.1	6	2	33.3	3	0	0
Autumn	1990	109	15	13.9	19	1	3.3	3	1	33.3
Spring	1991	28	4*	14.0	0	0	0	2	1	50.0
Total		275	55	20.0	39	6	15.4	16	5	31.2

a - number of hosts examined; b - number of hosts positive; c - infection rate (%) of *Cryptosporidium* sp.; (*) endogenous stages of *Cryptosporidium parvum* were found in the small intestine.

DISCUSSION

Cryptosporidium are coccidian parasites which have been recognized as a cause of diarrhoeal disease in man since 1976 (Meisel et al. 1976, Nime et al. 1976), and from that time a tremendous progress has been made in the studying epidemiology of cryptosporidiosis both in immunocompetent and immunodeficient persons. However, very little information is available on prevalence, significance and prognosis of the *Cryptosporidium* sp. infection in Poland (Siński et al. 1988). Our data has revealed that 2.4% of the children from the Memorial Children's Hospital in Warsaw, to which patients are referred from all districts of the country, were infected. Among groups of children ranging from 2 months to 17 years old, there was no correlation between prevalence of infection and age or sex. The age range for *Cryptosporidium* sp. infection in man has been previously reported from 3 days old (Bossen and Britt 1985) to 95 years of age (Holten-Anderson et al. 1984). Children from 12 to 35 months are believed to be at the highest risk for infection and our data confirms that *Cryptosporidium* sp. does occur in Poland mostly in children less then 3 years of age.

The source of infection for our patients has not been established. However, it is known that *Cryptosporidium* can be transmitted directly from animal to man, from man to man or indirectly via contaminated water or food. Studies within the past decade have clearly shown that calves are an important source of human infection (Anderson et al. 1982, Reese et al. 1982, Current et al. 1983). Furthermore, it has been suggested that also rodents are reservoir hosts (Klesius et al. 1986). Our data has revealed that 20% of rodents harbour *Cryptosporidium*. In the present study, apart from the estimation of the shedding of oocyst, in fact, in low concentration, it has been shown histologically that endogenous stages of *Cryptosporidium* were present in the small intestine. There is clear evidence that at least one species of wild rodents was infected with *C. parvum*. This preliminary result indicates that wild rodents and particularly *C. glareolus* may act as a reservoir for *C. parvum*. Thus, these animals should be considered as a potential source for ruminant and human cryptosporidiosis and it may be predicted that infection by this pathogen can be partly maintained by a mouse-cattle cycle.

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