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). Cryptosporidiosis are parasites of the alimentary and respiratory mucosae, invading the microvillus border of the enterocytes. Infections associated with diarrhea 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, Warsaw during a 5-year period. A total of 692 children (3 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, Urwitalt, near Mikolajki). 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...
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 con-
tents 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 dia-
rhoea. In one particular boy, a one-year-old boy, with
heavy diarrhoeal symptoms and with hypogammaglobu-
linemia 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 spo-
rozites. 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 pro-
duction were observed among children from villages
(43%) compared with groups from towns (21%) and citi-
es (36%).

Nine of all Cryptosporidium positive cases were asso-
ciated 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 scre-
ced for general immunocompetence. Four of these exhi-
bited 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 reve-
aled that of 330 examined animals 66 (20%) shed oocysts:
55 of 275 C. glareolus, 6 of 39 A. flavicollis, 5 of 16 S. ara-
neus were positive for oocysts of Cryptosporidium. The
prevalence of Cryptosporidium sp. infection in all exami-
ned 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
cparvum 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 ex-
amination, the endogenous stages of Cryptosporidium
were found in the upper part of the small intestine (duo-
denum and jejunum) in C. glareolus (Fig. 2). There was
no evidence of Cryptosporidium infection following both
coprosopic 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).

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

<table>
<thead>
<tr>
<th>Season</th>
<th>C. glareolus</th>
<th>A. flavicollis</th>
<th>S. araneus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>Autumn</td>
<td>1989</td>
<td>60</td>
<td>18*</td>
</tr>
<tr>
<td>Spring</td>
<td>1990</td>
<td>78</td>
<td>18</td>
</tr>
<tr>
<td>Autumn</td>
<td>1990</td>
<td>109</td>
<td>15</td>
</tr>
<tr>
<td>Spring</td>
<td>1991</td>
<td>28</td>
<td>4*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>275</td>
<td>55</td>
</tr>
</tbody>
</table>

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, Nimes 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 (Sinski 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 than 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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REFERENCES


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