

Age-Related Seroprevalence of *Cryptosporidium* in Northern Israel

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Abstract

Background: Two recent studies found that the prevalence of cryptosporidiosis among children in Israel was 3.4–7.4%.

Objectives: To assess the cumulative infection rate by testing immunoglobulin A and G seroprevalence for *Cryptosporidium* in children and adults in Israel.

Patients and Methods: The seroprevalence of IgA and IgG anti-*Cryptosporidium* antibodies was determined by an enzyme-linked immunosorbent assay procedure in a group of 163 healthy children and adults.

Results: The overall seroprevalence rates for IgG, IgA, both IgA and IgG, and any immunoglobulin were 12.6%, 23%, 30% and 65.6% respectively. Half the children under the age of 12 years were already infected, with seroprevalence increasing to 95.6% in those over age 13 ($P < 0.05$). Seropositivity for IgG or IgA did not significantly increase with age.

Conclusions: These results indicate that a large percentage of healthy children and adults in northern Israel have been infected with *Cryptosporidium*, and at early ages.

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Cryptosporidium parvum has been recognized worldwide as a common cause of diarrhea in otherwise healthy children. The prevalence of cryptosporidiosis has been shown to be 0.3–4.3% and 1.3–22% in children in developed and underdeveloped countries respectively [1]. Several seroprevalence studies have been performed to assess the extent of *Cryptosporidium* infection in children and adults. These studies documented cumulative infection rates of 31–32% and 64–82% in the United States and in Latin America respectively [2–5]. The aim of the present study was to assess the IgA and IgG cumulative seroprevalence rates for *Cryptosporidium* in children and adults in Israel.

Materials and Methods

The total population of the Jezre'el Valley in northern Israel is about 220,000 people, of whom 35% are Arabs and 65% are children under the age of 18 years. The study was approved by the Helsinki Committee and carried out in the

Microbiology Laboratory at the Central Emek Medical Center in Afula, which serves most of the population in this region.

We studied the sera of 27 healthy adults and 126 healthy children aged 1–18 years, which were sent to the laboratory by family physicians or pediatricians for various serologic tests. Sera were selected by age and origin of the person from whom it had been obtained in order to achieve maximum representation of the population of the Jezre'el Valley. The study population comprised 33% Arabs, and about two-thirds of both the Jewish and Arab subjects were children. None of the study population suffered from gastroenteritis when the sera were obtained.

The determination of IgA and IgG anti-*Cryptosporidium* antibodies was performed using an ELISA procedure as previously described, with several modifications in order to increase its accuracy [4–6]. The capture antigen consisted of oocytes that had been isolated from the stool of infected calves [4,5]. The oocyte suspension was then passed through three cycles of sugar flotation for purification and concentration of the oocytes. After purification, the oocytes were sonicated for 20 minutes. For IgA determination, sera were pretreated with rabbit antihuman IgG (Gull-Sorb, Gull Laboratories, USA) according to the manufacturer's recommendations. Serum of a person known to have been infected recently with *Cryptosporidium* was used as a positive control. As a negative control, we used a pool of sera obtained from healthy children under the age of one year living in northern Israel. All sera were run in duplicate. Sera and conjugates were diluted with 1% bovine serum albumin in phosphate-buffered saline. Washings were performed five times with PBS/Tween 0.05%.

The ELISA procedure was performed using ETI-LAB equipment (Sorin Biomedica, USA) with several modifications. For each procedure run positive and negative, controls were also run. Serum volumes of 50 μ l/well were used. Immulon II plates were coated with 60,000–70,000/well of sonicated oocytes in PBS. Plates were then incubated for one hour at room temperature and washed. Coated plates were blocked with 5% BSA in PBS and incubated for one hour at 37°C. Wells were then emptied but not washed. Sera

ELISA = enzyme-linked immunosorbent assay

PBS = phosphate-buffered saline

BSA = bovine serum albumin

diluted 1/100 were added to each well and plates were incubated for one hour at 37°C. Sera were then removed and plates were washed. Rabbit anti-human IgG or IgA conjugated with peroxides (DAKO) diluted 1/800 was added to each well. Plates were incubated for one hour at 37°C, emptied and washed. TMB was added to each well. Following 5 minutes incubation at room temperature, stopping solution was added to the wells and the optical density of the solutions was read at 450 μm. Negative control sera were run in five wells. Positive sera were defined as having an OD greater than the mean +3 SD OD of the negative controls. Statistical analysis was performed using Chi-square analysis. A *P* value <0.05 was considered significant.

Results

Age distribution and seroprevalence rates are shown in Table 1. Seropositivity for IgG or IgA was low and did not increase significantly with age. However, most of the study population had both IgG and IgA to *Cryptosporidium*. The combined IgA and/or IgG seroprevalence of children younger than 13 years was 50% and increased significantly to 95.6% in adolescents and adults. The overall seropositivity for *Cryptosporidium* was 65.6%. There was no statistically significant difference between the Jewish and the Arab populations in all age groups and in overall seroprevalence (60.4% and 67.1% respectively).

Table 1. Seroprevalence of *Cryptosporidium* in northern Israel

Age	No. of children	Positive IgG only	Positive IgA only	Both pos. IgG & igA	Any positive Ig
1-5	66	18.0%	19.6%	15%	52.6%
6-12	51	11.8%	25.5%	17.6%	50.9%
>13	46	4.6%	26%	65%	95.6%
Total	163	12.6%	23%	30%	65.6%
<i>P</i> *		NS	NS	<0.05	<0.05

* *P* values for significant distribution among all age groups

Discussion

Our study population is representative of the population in the Jezre'el Valley. The results of cryptosporidial seroprevalence show that half of the children and almost all of the adolescent and adults were infected by *Cryptosporidium*. In addition, there were no significant differences between the seroprevalence for *Cryptosporidium* in the Arab and the Jewish populations in all age groups.

The IgG seroprevalence rate in our population is lower than that of developing countries such as Peru and Venezuela, where rates of 64% and 58.6–81.8% respectively were found. In both countries 50–60% of the children at all ages had IgG antibodies to the parasite [2]. However, our findings resemble those of two recent U.S. studies. In a study conducted in Oklahoma, Kuhls et al. [4] studied the

combined IgA, IgG and IgM seroprevalence in children and young adults. The mean seroprevalence rate was 31%, and increased from 13% in children under 5 years old to 38% and to 58% in those aged 5–13 and 14–21 years old respectively. In our study, the mean combined IgG and IgA seroprevalence was 30% and increased from 15–17.5% in children to 65% in adolescents and adults (we did not study IgM seroprevalence). In the second study, in southern New England, the mean combined IgG and/or IgA seroprevalence rate in children and adults was 28.3%, increasing from 17–30% in children to 68.7% in adults [6].

In addition to determining IgG, we performed IgA serology to determine the prevalence of *Cryptosporidium* infection. *Cryptosporidium* is a gut-surface pathogen. In other enteric surface infections (such as rotavirus), IgA response is usually elicited as a part of the immune response and has a local protective effect [7,8]. Of note is the study by Casemore [9], who found that some individuals with cryptosporidiosis had serum anti-*Cryptosporidium* IgA without IgG or IgM response. In our study, seroprevalence rates for IgG and IgA were not concordant in most cases, emphasizing the importance of determining both antibodies in assessing the *Cryptosporidium* infection rate. In fact, in our study, 23% of the study population overall had *Cryptosporidium* IgA only. The *Cryptosporidium* seroprevalence rates in our study are higher than those found in the U.S. [4,6], suggesting that cryptosporidial infection is more common in Israel than in the USA.

Infected water is a major source of transmission of cryptosporidiosis. Other frequent modes of transmission are person to person, especially among children in day-care centers, and transmission from domestic animals (such as horses, calves, sheep, etc.) [10]. To our knowledge, no large studies on modes of transmission of cryptosporidial infection have been performed in Israel. However, the higher seroprevalence of *Cryptosporidium* in children and in almost all adults in Israel suggests multiple exposure to the parasite through contacts with any or all of these sources.

Our results show that infection by *Cryptosporidium* is highly prevalent in Israel and occurs early in life. Symptomatic cryptosporidiosis in Israel is most often seen in children. Dagan et al. [11] found *Cryptosporidium* in the stools of 13 of 382 children (3.4%) under the age of 3 years with diarrhea in southern Israel, as compared to 1 of 118 (0.7%) in a control group (*P*=0.078). In another study in northern Israel we demonstrated *Cryptosporidium* in the stools of 77 of 1,077 children (7.4%) younger than 14 years of age with diarrhea, compared to 2 of 90 (2.2%) in a control group (*P*<0.05). Cryptosporidiosis was more prevalent in children younger than 5 years than in older children (10.1% vs. 1.8% *P*<0.05). These results demonstrate a relatively high prevalence of childhood cryptosporidiosis in Israel. Also, it may explain the higher IgA than IgG seroprevalence in young children in our study.

In this as in other studies, the high seroprevalence rate is not in agreement with the low prevalence rate of

cryptosporidiosis. Since cryptosporidiosis may be asymptomatic or present as a mild self-limited disease, many parents do not seek medical aid for their children. In addition, stool testing for *Cryptosporidium* is not a routine procedure in most microbiology laboratories. Consequently, under-appreciation of infection with *Cryptosporidium* may occur. In summary, our serological findings indicate that a large percentage of healthy children and all adults in northern Israel have been infected with *Cryptosporidium*, and were infected at an early age.

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