

***Cryptosporidium* Infection in Bedouin Infants Assessed by Prospective Evaluation of Anticryptosporidial Antibodies and Stool Examination**

Guy Robin,¹ Drora Fraser,^{2,3} Nadav Orr,¹ Tamar Sela,¹ Raphael Slepon,¹ Ruhama Ambar,¹ Ron Dagan,⁴ Sylvie Le Blancq,⁵ Richard J. Deckelbaum,^{6,7} and Dani Cohen^{1,8}

An enzyme-linked immunosorbent assay system using oocyst lysate as antigen was used to detect serum-specific antibody responses to *Cryptosporidium parvum* between 1989 and 1994 in consecutive sera obtained at birth, and at the age of 6, 12, and 23 months, from 52 infants living in a Bedouin town located in the south of Israel. The serologic tests revealed high levels of immunoglobulin G anti-*Cryptosporidium* at birth that dropped significantly by the age of 6 months and then rose continuously to a geometric mean titer of 481 at age 23 months. The serum immunoglobulin M *Cryptosporidium* antibodies rose continuously from nearly undetectable levels at birth to a geometric mean titer of 471 (157-fold increase) at age 23 months. All the subjects already showed at 6 months a significant rise in immunoglobulin M. A significant rise in immunoglobulin A titers was detected in 48% and 91% of subjects at 6 and 23 months, respectively. By monthly surveillance, microscopy using the modified Ziehl-Neelsen method and confirmed by indirect immunofluorescence assay detected *Cryptosporidium* antigens in only 11% at age 6 months and 48% at age 23 months. The extent of exposure to *Cryptosporidium* immediately after birth as detected by serology is much higher than that predicted by frequent prospective assessment of stool samples. *Am J Epidemiol* 2001;153:194–201.

antibodies; *Cryptosporidium*; enzyme-linked immunosorbent assay; infant; infection

Cryptosporidium parvum is a coccidian protozoan parasite accepted as a pathogen of the digestive tract in humans. The infection is associated with diarrhea (1–6), and it is more prevalent in children who are malnourished (1, 5, 7, 8). In immunocompromised hosts, the impact of the disease is severe and includes respiratory problems, cholecystitis, hepatitis, and pancreatitis. On the other hand, in immunocompetent persons, infection is considered to be a self-limited disease, and the subclinical infection rate is unknown (3, 9, 10).

Cryptosporidiosis has a worldwide distribution. Prevalence varies from 1.3 percent in Scandinavian countries (11) to 16.7 percent in Haiti (12), and cryptosporidiosis spreads by person-to-person transmission as indicated by the occurrence of day-care center outbreaks (13, 14), multiple family infections (15), and sequential infections in hospitalized patients and personnel (16). Other ways of transmission include exposure to water (17, 18), animal contacts (19, 20), and travel (21).

Immunity to *Cryptosporidium* infection is believed to be conferred by cellular mechanisms that are at least in part antibody dependent (1, 19, 22, 23). The infection elevates specific antibody responses, and serology tests are potentially useful for the assessment of the extent of cryptosporidial infection in different populations. Infection has been described in all six continents, and specific anti-*Cryptosporidium* antibodies were found in 49.5 percent of examined children in endemic areas, such as three rural communities of Anhui (24), or in more than 60 percent of the sera obtained from subjects in two low socioeconomic populations in Peru and Venezuela (3). In general, the prevalence of *Cryptosporidium* infection was reported to be higher among children than among adults (24, 25).

A prospective study has been carried out in a cohort of Bedouin infants, during the first 2 years of life, to describe the natural history of infection with *Cryptosporidium* and to examine the association between *Cryptosporidium* infection and host and environmental risk factors (26). The Bedouin society, which is a society in transition from a nomadic to a settled semiurban lifestyle, can serve as a model for the study

Received for publication August 17, 1999, and accepted March 6, 2000.

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

¹Army Health Branch Research Unit, Medical Corps, Israel Defence Force, Military Post 02149, Israel.

²Epidemiology and Health Services Evaluation Department, Soroka University Medical Center, Beer-Sheva, Israel.

³Daniel Abraham International Center for Health and Nutrition, Soroka University Medical Center, Beer-Sheva, Israel.

⁴Pediatric Infectious Disease Unit, Soroka University Medical Center and Ben-Gurion University of the Negev, Beer-Sheva, Israel.

⁵Division of Environmental Health Services, School of Public Health, Columbia University, New York, NY.

⁶Department of Pediatrics, Columbia University, New York, NY.

⁷Institute of Human Nutrition, Columbia University, New York, NY.

⁸Department of Epidemiology and Preventive Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel.

Reprint requests to Dr. Dani Cohen, Army Health Branch Research Unit, Medical Corps, Israel Defence Force, Military Post 02149, Israel (e-mail: danic@netvision.net.il).

of parasitic infections in the high-risk groups of newly urbanized populations. Both frequent stool analyses (monthly) and serology were used to assess the extent of infection with *Cryptosporidium* among these infants in the first 2 years of life. We describe herein the seroconversion rates and kinetics of the *Cryptosporidium* serum antibodies among the Bedouin infants at 6, 12, and 23 months after birth and among their mothers immediately following delivery.

MATERIALS AND METHODS

Study location and study population

The serologic study was part of a prospective study carried out in a cohort of 234 Bedouin infants during the first 2 years of life and aimed to describe the natural history of infection with *Cryptosporidium* and to examine the association between *Cryptosporidium* infection and host and environmental risk factors (26). The infants under surveillance belonged to a community in transition from a nomadic to a settled semiurban lifestyle. The Bedouins were desert nomads living in tent encampments in family or tribal groups. Their migration was related to the grazing needs of their camels, sheep, and goats. The tribes now settled in Israel originated in various locations including the Middle East and North Africa. The study was conducted in a township located in the southern part of Israel, 30 km north of Beer-Sheva. The climate in this region is desert-like with temperatures ranging from 10°C to 40°C. For 4 months of the year, usually from mid-May through mid-September, peak day temperatures do not fall below 30°C. On average, it rains only 24 days a year, mainly between November and March.

Intake and follow-up procedures

During the period from November 1989 to December 1992, 234 healthy infants from the Bedouin town were recruited after obtaining informed consent to the study. Fifty-two of the infants, born in 1992, and their mothers were included in the serologic study. Maternal blood samples were obtained from routine postdelivery blood samples. Cord bloods from consenting mothers were retained. A pediatric examination was performed at the clinic at birth and at the ages of 6, 12, and 23 months, at which consecutive blood samples were drawn, separated into plasma, and frozen until tested. Blood samples were available from all the 52 mothers at the time of delivery. A total of 52, 48, 49, and 40 serum samples were obtained at birth and at 6, 12, and 23 months, respectively, from the 52 infants included in the serologic follow-up. From November 1989 to December 1992, surveillance for diarrheal disease was carried out routinely on arrival of the infants at the primary health clinic, at the emergency room of the hospital, or at the Maternal and Child Health clinics where study nurses were stationed. This morbidity surveillance system was supplemented by maternal reports at monthly home visits. From January 1993 to the end of the study (July 1994), morbidity was ascertained weekly by interviewers trained to inquire about diarrhea by a home visit (85 percent of homes) or by telephone inter-

view (15 percent of homes) and by information obtained at the monthly home visit. Nurses and pediatricians examined the infants, and diarrheal episode forms were completed. Stool samples were obtained monthly as well as at all new episodes of diarrhea and 7- to 10-day intervals thereafter until the episode ended. Of the samples for collection during routine surveillance (at monthly home visits), 93.2 percent were obtained. During diarrheal episodes, 73.0 percent of the samples that should have been obtained were collected. The stool samples were examined routinely for the following parasites and bacterial enteropathogens: *C. parvum*, *Giardia lamblia*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., enteroaggregative *Escherichia coli*, diffuse adherent *E. coli*, localized adherent enteropathogenic *E. coli*, enterotoxigenic *E. coli* expressing heat-stable toxin, enterotoxigenic *E. coli* expressing heat-labile toxin, enterotoxigenic *E. coli* expressing both toxins, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, and *Rotavirus*.

Stools were collected from diapers or directly into a disposable cup, transferred to tubes containing phenol-alcohol-formaldehyde (27), and transported on ice to the Parasitology Laboratory at Soroka University Medical Center. *Cryptosporidium* was detected by the modified Ziehl-Neelsen method and confirmed by indirect immunofluorescence assay using the Merifluor *Cryptosporidium* indirect immunofluorescence detection procedure (Meridian Diagnostics, Inc., Cincinnati, Ohio).

Serologic methods

An enzyme-linked immunosorbent assay was performed in polystyrene microtiter plates (model 3590; Costar, Cambridge, Massachusetts) according to the method of Ungar et al. (28) with some modifications. Briefly, 100 µl of coating buffer (0.05 M carbonate buffer, pH 9.6) containing lysate of 10⁴ calf oocysts after 20 freeze-thaw cycles were added to each of 96 wells, and the plates were incubated for 1 hour at 37°C. After removal of the coating solution, the plates were incubated for 1 hour at 37°C with 0.05 M phosphate-buffered saline supplemented with casein and bovine serum albumin (both at 5 g/liter). The plates were then washed twice in phosphate-buffered saline-Tween 20 (Pierce Chemical Company, Rockford, Illinois) washing solution. Sera were diluted in blocking buffer (1:25 for immunoglobulin G (IgG) and immunoglobulin M (IgM) and 1:5 for immunoglobulin A (IgA)) and added to the first line of wells in the microtiter plates. The sera were then double diluted seven times in blocking buffer and incubated overnight at room temperature. After four additional washings, goat anti-human immunoglobulin G, A, or M conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) was added to the wells. The plates were incubated overnight at room temperature and washed, and an enzyme-linked immunosorbent assay was completed by the addition of the enzyme-substrate solution containing *para*-nitrophenylphosphate (1 mg/ml) in diethanolamine buffer at pH 9.8. The reactions were stopped with 3 M NaOH, and absorbance was read at 405 nm with an automatic enzyme-linked immunosorbent assay biokinetics EL340 reader (Bio-Tek Instruments,

Winooski, Vermont). The consecutive serum samples of each subject were tested within the same assay, and positive and negative control sera were included in every microtitration plate in each of the assays. The adjusted absorbances derived from a linear regression analysis of eight doubling dilutions were expressed as endpoint titers (at absorbance = 0.3), and geometric mean titers were calculated.

The specificity of the enzyme-linked immunosorbent assay system used in the study was documented in two different ways.

Lack of cross-reactivity in paired sera of subjects exposed to bacterial enteropathogens. The same criterion for significant antibody response to *Cryptosporidium* (equal to or greater than a fourfold rise in titer) was used to detect a potential nonspecific rise in serum *Cryptosporidium* antibodies in pairs of sera ($n = 10$) obtained 14 days apart from healthy adult subjects vaccinated with an investigational enterotoxigenic *E. coli* vaccine and in pairs of sera obtained from cases of laboratory-proven *Shigella sonnei* ($n = 5$), *Shigella flexneri* ($n = 5$), *Salmonella typhimurium* ($n = 5$), and enterotoxigenic *E. coli* ($n = 5$) infections. The anti-*Cryptosporidium* titers in these paired sera were extremely stable. The ratios of the antibody titers between post- and preenterotoxigenic *E. coli* vaccination or post- and pre-*Shigella*, *Salmonella*, and enterotoxigenic *E. coli* infections ranged between 0.8 and 1.5.

Absorption study. One hundred μl of a 1:400 dilution of each of five sera with high IgG *Cryptosporidium* antibody levels were mixed with 100 μl of 5×10^5 oocysts and incubated for 2 hours, rotating at 37°C. The mixtures were then centrifuged at 13,000 rpm for 10 minutes. Eight twofold dilutions of the absorbed and unabsorbed sera were tested in the same enzyme-linked immunosorbent assay microplate for the presence of *Cryptosporidium* antibodies. A significant reduction of 47–62 percent of the IgG *Cryptosporidium* geometric mean titer was measured in the absorbed as compared with the unabsorbed corresponding sera.

Definitions and data analysis

An episode of diarrhea was defined as the passing of loose stools at least four times in a 24-hour period for infants less than 1 month old and at least three times in a 24-hour period for older children. A new episode of diarrhea was preceded by three diarrhea-free days. Seroconversion was defined as an antibody response equal to or greater than a fourfold rise in titer compared with that of day 0, while the second serum sample had to reach an antibody titer higher than a threshold value. The threshold titers were defined in order to screen out very small rises that still meet the fourfold criterion. The values chosen were based on the mean baseline titer plus 2 standard deviations (baseline titer was that of age 0 for IgA and IgM and that of age 6 months for IgG). The threshold titers were 333, 17, and 17 for IgG, IgM, and IgA, respectively. The statistical significance for comparison of geometric mean titers at various times was calculated using Duncan's multiple range tests. The statistical significance for comparison of seroconversion rates was calculated using chi-square or Fisher's exact tests.

RESULTS

The Bedouin families of the 52 infants included in the serologic study come from a variety of tribal groups. The characteristics of these infants and of their families were similar to those of the entire cohort of 234 infants and families. At the time of enrollment, 96 percent of them lived in brick houses. Four percent of the families still lived in huts or in the traditional Bedouin tents. Outdoor water pipes supplied 8 percent of the dwellings, 2 percent had no electricity, and 16 percent had outdoor toilets. The mean maternal age was 25.4 (standard deviation, 6.3) years. The mothers and fathers of the 52 infants had 5.8 (standard deviation, 4.2) years and 8.0 (standard deviation, 4.5) years of schooling, respectively. At the time of enrollment, the index child was the only child in 32 percent of the families; 50 percent of the families had 1–4 additional children, and the remaining families (18 percent) had five or more additional children. The mean age at which infants were weaned was 10.3 (standard deviation, 6.2) months.

The experience with diarrheal episodes was similar among the 52 infants included in the serologic study and the rest of the cohort. There were no significant differences in the total number of the diarrheal episodes, their distribution during the 2 years of follow-up, the length of the episodes, and the infants' age at which the first episode occurred. Almost all of the 52 infants who were followed up from birth to the age of 2 years experienced at least one episode of diarrhea during this period. Twenty percent of them had seven or more episodes, and 40 percent had suffered from 3–6 events.

C. parvum was identified in 25 (48 percent) of the 52 infants during the 23-month follow-up period. Eleven of the infants with *Cryptosporidium* were symptomatic, and 14 were asymptomatic when the parasite was detected. The timing of *Cryptosporidium* detection in the 25 infants was evenly distributed throughout the 23 months of follow-up: six infants were positive in the first 6 months of life, another six, between 7 and 12 months, and the rest of 13 infants harbored *Cryptosporidium* between the age of 13 and 23 months. Five of the 25 infants shed *C. parvum* in two or three consecutive stool samples for at least 9–17 days. These were the only subjects with multiple *Cryptosporidium* infections. *Cryptosporidium* was detected as a single pathogen among 17 of the 25 infants with positive stool samples, while the other *Cryptosporidium*-infected infants were coinfecting with the following enteropathogens: *G. lamblia* (three infants), *G. lamblia* and enterotoxigenic *E. coli* expressing heat-stable toxin (one infant), *Rotavirus* and enteroaggregative *E. coli* (one infant), diffuse adherent *E. coli* (one infant), enterotoxigenic *E. coli* expressing heat-labile toxin (one infant), and *Campylobacter* spp. (one infant). The serologic tests carried out on the consecutive serum samples obtained from the 52 infants revealed an IgG anti-*Cryptosporidium* geometric mean titer of 339 (95 percent confidence interval: 290, 413) at birth, most probably of maternal origin (figure 1). The geometric mean titer of the mothers of the same infants using the postdelivery samples was 1,295 (95 percent confidence interval: 1,123, 1,486). The level of IgG antibodies dropped significantly by

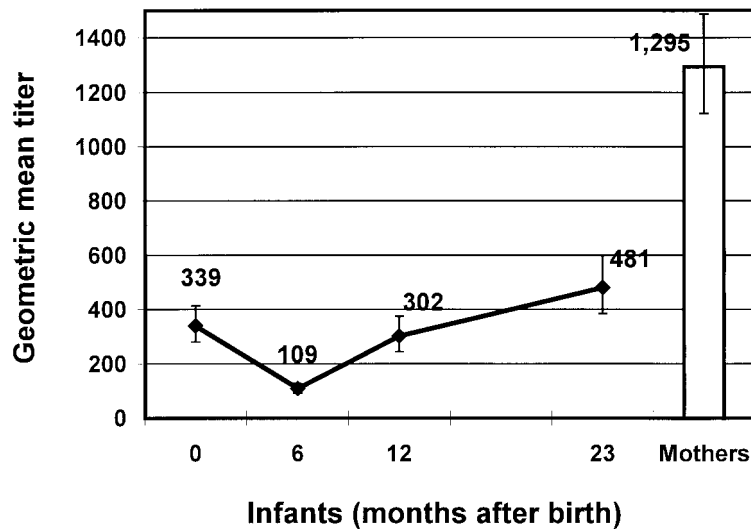


FIGURE 1. *Cryptosporidium* immunoglobulin G antibodies (expressed as geometric mean titer) in 52 Bedouin infants at different times after birth and among the infants' mothers immediately after delivery, Israel, 1989–1994. All differences are statistically significant ($p < 0.05$) by Duncan's multiple range test. Bars, 95% confidence interval.

the age of 6 months and then rose continuously to the level of 481 (95 percent confidence interval: 385, 598) at the age of 23 months, when it reached less than half the titer measured in mothers.

The serum IgM *Cryptosporidium* antibodies rose continuously from a barely detectable level at birth to a geometric mean titer of 102, 272, and 471 at age 6, 12, and 23 months, respectively (figure 2). There was no significant difference in the geometric mean titer of IgM antibodies measured at 6, 12, and 23 months between infants with a low IgG titer (<303) as compared with infants with a high IgG titer (≥ 303) of specific antibodies estimated at birth (106, 258,

and 435 vs. 111, 345, and 525, respectively). The IgM titer measured among mothers at the timing of delivery was twice as high as that measured in infants at the age of 23 months.

The titers of serum IgA *Cryptosporidium* antibodies produced by the infants increased significantly throughout the first 2 years of life but were much lower than those of IgM and IgG immunoglobulins (figure 3). As was found for IgG and IgM *Cryptosporidium* antibodies, the IgA geometric mean titer of 487 (95 percent confidence interval: 370, 641) measured in the serum samples obtained from the infants' mothers postdelivery was significantly higher than that

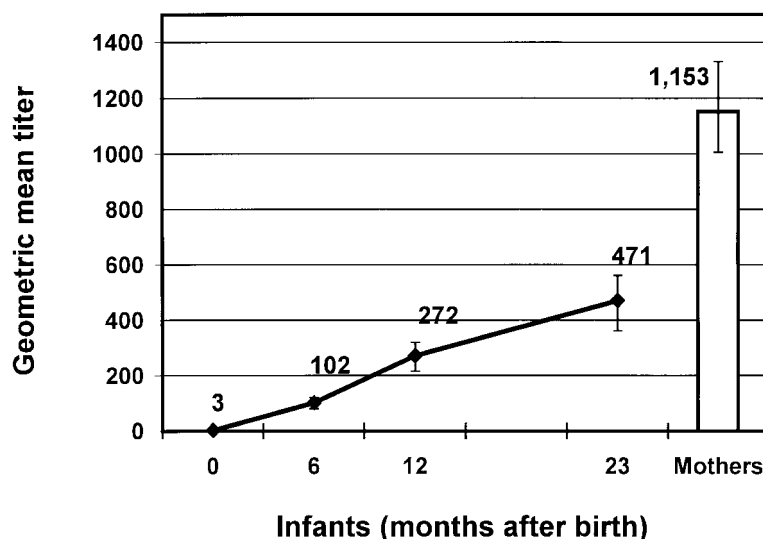


FIGURE 2. *Cryptosporidium* immunoglobulin M antibodies (expressed as geometric mean titer) in 52 Bedouin infants at different times after birth and among the infants' mothers immediately after delivery, Israel, 1989–1994. All differences are statistically significant ($p < 0.05$) by Duncan's multiple range test. Bars, 95% confidence interval.

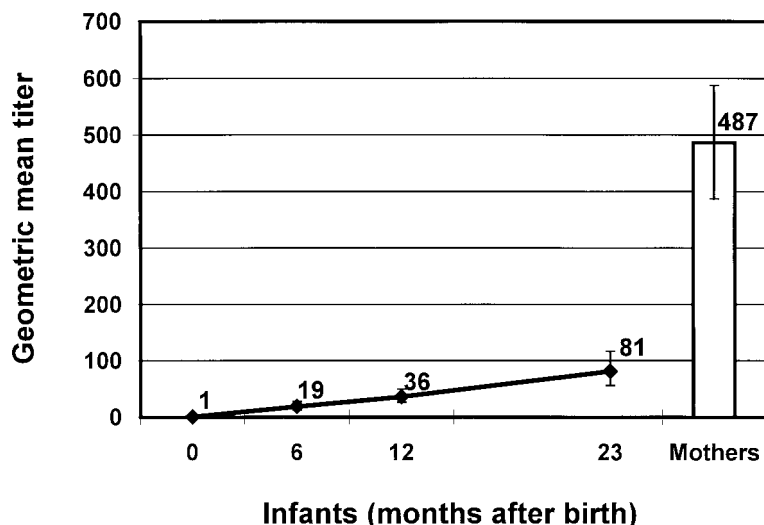


FIGURE 3. *Cryptosporidium* immunoglobulin A antibodies (expressed as geometric mean titer) in 31 Bedouin infants at different times after birth and among the infants' mothers immediately after delivery, Israel, 1989–1994. All differences are statistically significant ($p < 0.05$) by Duncan's multiple range test. Bars, 95% confidence interval.

detected in the sera of the infants at the age of 23 months. Seroconversion rates of 48 percent and 100 percent in the IgA and IgM titers were already detected at the age of 6 months, and 42 percent of the infants showed a significant serum IgG response to *Cryptosporidium* between sera obtained at the ages of 6 and 23 months (figure 4). No significant difference in the pattern and magnitude of the serum antibody response of IgG, IgA, and IgM to *Cryptosporidium* was detected in the consecutive sera of infants with ($n = 25$)

and without ($n = 27$) laboratory-proven *Cryptosporidium* infection detected by stool analyses. No significant difference in the pattern and magnitude of the serum antibody response to *Cryptosporidium* was detected among infants harboring the homologous parasite alone ($n = 17$) or together with other enteropathogens ($n = 8$).

When the 52 infants were divided into two groups with IgG *Cryptosporidium* antibody levels at birth higher or lower than the median titer, we found during the 23 months

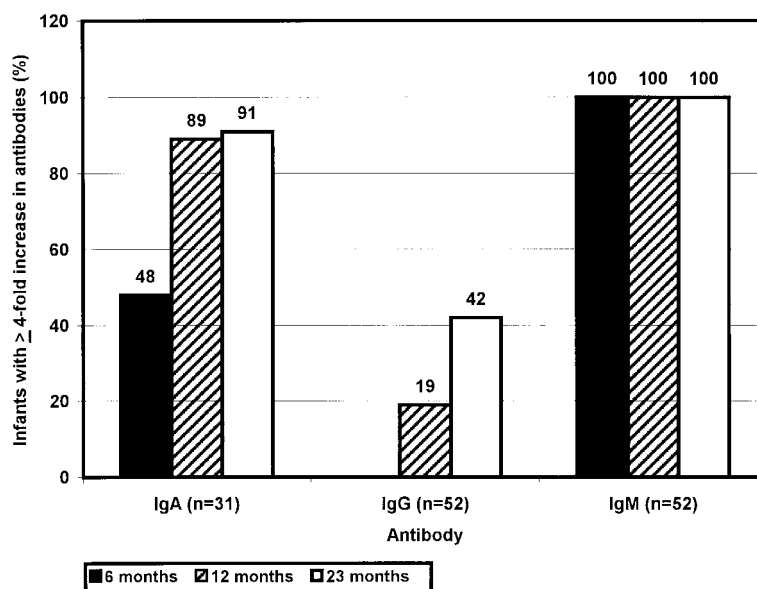


FIGURE 4. Immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) seroconversion rates to *Cryptosporidium* at 6 (solid black), 12 (striped pattern), and 23 (white outlined in black) months after birth among Bedouin infants, Israel, 1989–1994. A ≥ 4 -fold antibody rise is shown, with the second titer larger than the cutoff value (mean baseline (standard deviation, 2)). Baseline levels are those from time 0 for IgA and IgM and those from time 6 months for IgG.

of follow-up a *Cryptosporidium* infection rate 50 percent higher among the infants with low titers (<303) than among those with high titers (≥ 303) (15/26 (57.6 percent) vs. 10/26 (38.5 percent)). The difference, however, did not reach statistical significance ($p = 0.16$).

DISCUSSION

The prospective serologic evaluation of 52 infants in the Bedouin community provided an opportunity to follow changes in the immune response to *Cryptosporidium* antigens from birth till the age of 2 years. The high rates of seroconversion to *Cryptosporidium* shortly after birth indicate a large amount of natural exposure to this coccidian protozoan in the Bedouin community in the south of Israel. Serology is much more sensitive than stool examination for the evaluation of exposure to *Cryptosporidium*. The serologic data revealed that by the age of 6 months all the infants under follow-up have already been infected with *Cryptosporidium* while, during the entire 23-month follow-up, only half of them were detected as positives for *Cryptosporidium* by at least monthly parasitologic examination of their stool samples. A similar ratio of serologically to parasitologically proven *Cryptosporidium* infections has been reported by cross-sectional and cohort studies in infants living in an urban slum area of Fortaleza, Brazil (24).

The specificity of the enzyme-linked immunosorbent assay system used in this study was documented by the lack of cross-reactivity with *Cryptosporidium* antigens of paired sera obtained from subjects exposed to various bacterial enteropathogens and by the significant reduction of the IgG *Cryptosporidium* geometric mean titer after absorption with *Cryptosporidium* oocysts. The specificity of the enzyme-linked immunosorbent assay using *Cryptosporidium* oocysts as antigens was previously addressed by others (1, 24). It has been shown that IgG binding to solid-phase adsorbed *Cryptosporidium* oocysts was preferentially inhibited by preabsorption with *Cryptosporidium* antigens but not with any of the following formalin-fixed organisms: *G. lamblia*, *Trichomonas vaginalis*, *Campylobacter jejuni*, enterotoxigenic *E. coli*, and *Candida albicans* (1). In a manner similar to that of our study, two other studies (1, 24), after one cycle of absorption with *Cryptosporidium* antigens, reported that an approximate 50 percent inhibition of specific antibody binding was achieved. A possible explanation for the limited inhibition is that more epitopes are exposed when the crude *Cryptosporidium* antigens are adsorbed to a solid phase because of partial denaturation as compared with the similar antigens in a suspension. In another study (29), however, it was shown that the sera of lambs or rabbits that were experimentally immunized with *Cryptosporidium* oocytes cross-reacted in indirect immunofluorescence assay and Western blot with various ovine *Eimeria* spp. In that study the investigators did not detect cross-reactivity with *Toxoplasma gondii* or *Sarcocystis gigantea* (29).

No significant association was found between the rate or magnitude of the immune response to *Cryptosporidium* during the first 2 years of life and detection of *Cryptosporidium* in stools from diarrhea- or nondiarrhea-associated samples.

These findings suggest that the immune system of the infants immediately after birth is sensitive to even a very low level of *Cryptosporidium* antigenic stimuli and promptly reacts with a specific serum response. It has been shown that a very low infectious dose can cause symptomatic *Cryptosporidium* infection (30, 31). It is conceivable that a subclinical immune response may be induced by exposure to even lower doses of *Cryptosporidium* oocysts.

The much higher titers of antibodies among Bedouin mothers, as compared with the Bedouin infants at the age of 2 years, are most probably a result of cumulative natural boosters following repeated exposure to *Cryptosporidium* in the community of Bedouins living in a township in the northern Negev. At birth, the infants had about a third of the level of anti-*Cryptosporidium* IgG measured in the sera of their mothers, suggesting that maternal IgG anti-*Cryptosporidium* passes through the placenta. The geometric mean titer of IgG *Cryptosporidium* antibodies decreased threefold from the levels measured at birth to those measured 6 months later, reflecting most probably the decay of antibodies of maternal origin. The continuous increase in the levels of serum IgM and IgA *Cryptosporidium* antibodies during the first 6 months of life suggests that the presence of maternal IgG did not interfere with the recognition of *Cryptosporidium* antigens by the infants' immune system.

Most of the serologic data related to *Cryptosporidium* infections have been reported in prevalence studies (1, 3, 6, 8, 28, 32–34) that showed, in general, a higher prevalence rate of *Cryptosporidium* antibodies in populations living in developing as compared with developed countries. Ungar et al. (28) showed that 20 percent of Ecuadorian children with diarrhea were seropositive for *Cryptosporidium*-specific IgM and IgG. Another study revealed that *C. parvum*-specific IgG was present in more than 60 percent of Peruvian children older than 2 years of age and in more than 50 percent of Venezuelan children, including those in the first year of life (3). A serologic study carried out among institutionalized Thai children aged 2–60 months, living under crowded conditions with steady contact among ages, showed elevated levels of *Cryptosporidium* antibodies in virtually all the 35 subjects examined (1). Similar to our data, this study did not reveal any difference in the *Cryptosporidium* antibody levels between subjects harboring or not harboring the parasite. Another seroprevalence study carried out among 803 children in Oklahoma showed that the seroprevalence of *C. parvum* antibodies was positively associated with the age of the children and negatively associated with their socioeconomic status (6). The seroprevalence rates in this population were 13 percent, 38 percent, and 58 percent for the age groups <5 years, 5–13 years, and 14–21 years, respectively (6). Seroprevalence studies carried out in adult populations showed the presence of detectable *Cryptosporidium* IgG antibodies in 32 percent of Peace Corps volunteers before they traveled to developing countries (8). Another study reported a *Cryptosporidium* IgG seroprevalence rate of 36 percent among adults in a farming community in Wisconsin (33). These studies defined seropositivity as an antibody level above a cutoff

point derived from the measurement of detectable specific antibodies in "negative controls" considered as healthy subjects without any documented previous exposure to *Cryptosporidium*. Because the negative controls were different in the various studies, the comparisons drawn between the rates of seroprevalence reported are of limited value. Our study, in which the infants were followed up serologically from birth till the age of 2 years, clearly reveals that very early after birth infants naturally exposed to *Cryptosporidium* elicit significant rises in the specific *Cryptosporidium* antibody titers. The increase in *Cryptosporidium* antibody levels during the follow-up period reflects most probably cumulative exposures and repeated antigenic stimuli. Similarly, it has been recently shown that the IgG seroconversion rate increased among volunteers exposed to consecutive challenges, 1 year apart, with *Cryptosporidium* (30, 35, 36). On the second challenge, the 50 percent infective dose for volunteers with pre-existing IgG antibodies (by enzyme-linked immunosorbent assay) was found to be 20-fold higher than that for antibody-negative volunteers, indicating that anti-*C. parvum* IgG correlates with protection to exposures to low numbers of the parasite (36). In our study, although without reaching statistical significance, a trend of an excess of parasitologically proven *Cryptosporidium* infections occurred during the 2-year follow-up among infants with low specific IgG titers measured at birth. It is not clear yet what will be the level of specific serum antibodies that could be associated with naturally acquired immunity to *Cryptosporidium* and whether the serum antibodies play an active role in mechanisms of immunity or are just a surrogate marker of another effector. It is believed that mechanisms of cell-mediated immunity are of importance in protection against cryptosporidiosis (22, 23, 34), but it was also shown that the specific *Cryptosporidium* serum antibodies could be also involved in protection against recurrent disease, possibly as part of an antibody-dependent cell-mediated cytotoxic effect (22, 23, 34).

In summary, the findings of the study indicate that Bedouin infants are naturally exposed to *Cryptosporidium* soon after birth. Serology was more sensitive than frequent prospective assessment of stool samples in detecting *Cryptosporidium* infections. The higher titers of antibodies among Bedouin mothers as compared with the young children at the age of 2 years may be a result of more natural boosters following repeated exposure to *Cryptosporidium*. It may also be possible that this difference reflects at least in part the immaturity of the infants' immune system.

ACKNOWLEDGMENTS

This study was supported by grant 1 PO1-AI-26497 from the National Institute of Allergy and Infectious Diseases-International Collaboration in Infectious Diseases Research, National Institutes of Health, Bethesda, Maryland.

The authors thank Dr. Cynthia Chappell for critical reading of the manuscript and useful discussions.

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