

THE INFECTIVITY OF *CRYPTOSPORIDIUM PARVUM* IN HEALTHY VOLUNTEERS

HERBERT L. DUPONT, M.D., CYNTHIA L. CHAPPELL, PH.D., CHARLES R. STERLING, PH.D.,
PABLO C. OKHUYSEN, M.D., JOAN B. ROSE, PH.D., AND WALTER JAKUBOWSKI

Abstract Background. Small numbers of *Cryptosporidium parvum* oocysts can contaminate even treated drinking water, and ingestion of oocysts can cause diarrheal disease in normal as well as immunocompromised hosts. Since the number of organisms necessary to cause infection in humans is unknown, we performed a study to determine the infective dose of the parasite in healthy adults.

Methods. After providing informed consent, 29 healthy volunteers without evidence of previous *C. parvum* infection, as determined by the absence of anti-cryptosporidium-specific antibodies, were given a single dose of 30 to 1 million *C. parvum* oocysts obtained from a calf. They were then monitored for oocyst excretion and clinical illness for eight weeks. Household contacts were monitored for secondary spread.

Results. Of the 16 subjects who received an intended dose of 300 or more oocysts, 14 (88 percent) became infected. After a dose of 30 oocysts, one of five subjects (20 percent) became infected, whereas at a dose of 1000 or more oocysts, seven of seven became infected. The median infective dose, calculated by linear regression, was 132 oocysts. Of the 18 subjects who excreted oocysts after the challenge dose, 11 had enteric symptoms and 7 (39 percent) had clinical cryptosporidiosis, consisting of diarrhea plus at least one other enteric symptom. All recovered, and there were no secondary cases of diarrhea among household contacts.

Conclusions. In healthy adults with no serologic evidence of past infection with *C. parvum*, a low dose of *C. parvum* oocysts is sufficient to cause infection. (N Engl J Med 1995;332:855-9.)

CRYPTOSPORIDIUM PARVUM is a coccidian parasite of many animal species. The agent is a common cause of diarrhea in virtually all human populations, and it has a particular predilection for certain hosts, including those who have contact with animals, patients with the acquired immunodeficiency syndrome, infants attending day-care centers, international travelers, and persons living in tropical regions of the developing world.¹⁻⁶ The infection represents one of the recently defined, emerging microbial threats to the population.⁷

Until recently, it was not clear how susceptible persons acquired the infection. It is now known that the organism can be contracted from young animals (especially calves), infected persons, or drinking water. Treatment and filtration of water lower the numbers of organisms in water,⁸⁻¹⁰ yet *C. parvum* oocysts can often be found in low levels in treated drinking water.¹¹ Furthermore, cryptosporidium oocysts are not killed by disinfectants and chlorination.^{10,12-14} Thus, drinking water may be an important source of human infection. Contamination of municipal water sources has resulted in large community outbreaks of cryptosporidiosis, even when the quality of the water met water-treatment standards.¹⁵⁻¹⁷

The present study was carried out to determine the infective dose of *C. parvum* in healthy subjects without serologic evidence of prior infection.

Four experts on cryptosporidium and a clinical im-

munologist-allergist were consulted, and all agreed that the study could be performed safely provided there was adequate screening of the volunteers to ensure that all were healthy. Several authorities from the Environmental Protection Agency reviewed the proposal and made suggestions that were incorporated into the study. The project was also reviewed and approved by a site-visit team from the National Institutes of Health. The data from this study and future studies will be used to determine the virulence of various strains of *C. parvum* and the resultant immunity after exposure, to estimate the risk of water-borne infection by this parasite with a method used previously for *Giardia lamblia*,¹⁸⁻²⁰ and to help reevaluate the adequacy of current standards for safe drinking water.

METHODS

The organism given to the subjects was originally isolated from a calf in Iowa and was propagated at the University of Arizona.²¹ One-day-old Holstein calves were infected with 200 million *C. parvum* (Iowa strain). Calf feces were collected and sieved sequentially through stainless-steel screens with a final pore size of 63 μm (230 mesh). Oocysts were isolated with the use of discontinuous sucrose and isopycnic Percoll gradients.²¹ Purified oocysts were stored in 2.5 percent potassium dichromate at 4°C before shipment by overnight delivery service to the University of Texas in Houston.

Two lots of the inoculum were cultured on artificial medium for aerobic and anaerobic bacteria, and one lot was tested in cell-culture lines to detect adventitious viral agents. For these studies, oocysts were inoculated onto the following cultures: phytohemagglutinin-stimulated, primary human leukocytes, with subsequent p24 antigen enzyme-linked immunosorbent assay (ELISA) for human immunodeficiency virus type 1; BGM monolayers to identify the cytopathogenic effect of enteroviruses; MDCK cell cultures to identify the cytopathogenic effect of myxoviruses; and guinea-pig erythrocytes to detect hemadsorption of myxoviruses. One lot of the inoculum was examined for viral particles by negative-staining electron microscopy at the Environmental Protection Agency. Before electron-microscopical examination, the oocysts were ruptured by three freeze-thaw cycles. The preparation was stained with 2 percent phosphotungstic acid, and two optimally stained grids were examined with a transmission electron microscope (JEOL 100 CX, JEOL USA, Peabody, Mass.). With this method the limit of detection is 1 million viral particles per milliliter.

The viability of oocysts in each lot was evaluated by in vitro excys-

From the University of Texas Medical School (H.L.D., P.C.O.) and the University of Texas School of Public Health (H.L.D., C.L.C.), Houston; the University of Arizona, Tucson (C.R.S.); the University of South Florida, Tampa (J.B.R.); and the Environmental Protection Agency, Cincinnati (W.J.). Address reprint requests to Dr. DuPont at St. Luke's Episcopal Hospital, 6720 Bertner Ave., MCI-164, Rm. P153, Houston, TX 77030.

Supported by a Cooperative Agreement with the Environmental Protection Agency (CR-819814), by a grant from the National Institutes of Health (MOIRR-02558), and by the King Ranch Family Foundation.

Portions of the study were presented at the Clinical Research Meetings, Baltimore, May 1, 1994; the Society of Protozoologists Meeting, Cleveland, June 24, 1994; and the Workshop on Prevention and Control of Waterborne Cryptosporidiosis, Centers for Disease Control and Prevention, Atlanta, September 22, 1994.

tation.²² An aliquot of each lot remained at the University of Arizona and was retested within three days of each challenge to determine the excystation rate. The viability of four lots of oocysts 30 days after isolation was determined on the basis of the inclusion (viable) or exclusion (nonviable) of the fluorogenic vital dye 4',6-diamidino-2-phenylindole.²³ In addition, the infectivity of the oocysts was ascertained in mice with the use of methods described previously.⁹ For these studies, an initial dose of 60 oocysts per mouse (based on previous studies of the mean infective dose [ID₅₀]) was bracketed by four additional doses in quarter-log increments. The mice were killed seven days after inoculation, and approximately 3 cm of the terminal ileum was removed, fixed in 5 percent formalin, embedded, and sectioned. Sections stained with hematoxylin and eosin were examined microscopically for evidence of intracellular parasites.

For each lot of oocysts, the date of production, the excystation rate, and the date of inoculation were recorded in Houston. To determine the effect of shipping on the viability of oocysts, the excystation rate of one lot was measured at the University of Arizona before shipment. When the oocysts were received in Houston, an aliquot was removed and immediately returned to the University of Arizona for a second measurement of the excystation rate. Before being given to the subjects, each lot of oocysts received in Houston was washed three times in 10 ml of sterile phosphate-buffered saline, pH 7.2. The oocysts were then resuspended in phosphate-buffered saline, subjected to serial 10-fold dilutions, and counted a minimum of three times with a hemacytometer. The number of oocysts was initially confirmed by direct immunofluorescence assay with an anti-oocyst monoclonal antibody (Merifluor Cryptosporidium/Giardia Direct Immunofluorescent Detection Procedure, Meridian Diagnostics, Cincinnati). On the basis of the mean value, an additional dilution was performed, if necessary, to produce the concentration needed for inoculation. Each inoculum was counted three to six times to confirm the number of oocysts. Various concentrations of cryptosporidium oocysts (30 to 1 million) were placed in gelatin capsules and given to the subjects with 250 ml of buffered saline within one hour of preparation. No other food or beverages were ingested 90 minutes before or after ingestion of the gelatin capsule. The subjects were studied in groups of three to six between March 1993 and January 1994.

Screening and Enrollment of Subjects

The volunteers for the study consisted of students and members of the administrative and research staffs at the Texas Medical Center in Houston, and they were identified by the University of Texas—Hermann Hospital Clinical Research Center; none worked for or were students of the investigators. Before enrollment, the subjects were given a thorough and detailed explanation of the study, as well as general information about *C. parvum* and cryptosporidiosis. Eligible volunteers were required to score 100 percent on a 10-question examination that tested their comprehension of salient features of the study, including the fact that they might become ill, that no effective treatment for the illness was available, and that the organism could be spread to household contacts. Subjects were excluded if their households included an infant, an elderly person, or someone who was chronically ill. Household contacts were also informed of the study.

The subjects were required to read and sign a consent form, provide a history, and pass a physical examination. The following studies had to be normal: complete blood count, blood chemistry panel, urinalysis, chest radiography, electrocardiography, stool tests for occult blood and parasites, tests for T-cell subgroups and immunoglobulins, and serologic studies for hepatitis B surface antigen, syphilis, and human immunodeficiency virus. In addition, women of childbearing age had to have a negative pregnancy test. The subjects were also required to have delayed skin reactivity to positive control antigens (trichophyton, mumps, and candida) and negative tuberculin skin tests. Only subjects who were negative for *C. parvum* on ELISA were eligible. The study was approved by the University of Texas Committee for the Protection of Human Subjects.

Anticryptosporidium Antibody Assay

The procedures for the anticryptosporidium antibody assay were adapted from those of Ungar et al.²⁴ Briefly, microtiter wells (Nunc-Immuplate, Nunc, Roskilde, Denmark) were coated with 0.2 µg of antigen prepared as previously described²¹ and allowed to bind over-

night at 4°C. The wells were then blocked by the addition of 5 percent dry milk in phosphate-buffered saline containing 0.1 percent polysorbate 20 (Tween 20) (Sigma Chemicals, St. Louis), followed by incubation overnight at 4°C or for one hour at 37°C. Between each step the plates were washed a minimum of three times with phosphate-buffered saline plus 0.1 percent Tween 20. Serum samples were diluted 1:2 in phosphate-buffered saline, and 50-µl aliquots were incubated in duplicate wells for one hour at 37°C. Positive- and negative-control serum samples were included in triplicate on each plate. The samples were washed, and 50 µl of a 1:1000 dilution of peroxidase-conjugated antihuman IgG or antihuman IgM was added to each well and incubated for one hour at 37°C. The plates were then washed six times before the addition of 50 µl of peroxidase-activated 2,2'-azino-di-[3-ethylbenzthiazolinesulfonate-(6)] (Boehringer Mannheim, Indianapolis). The plates were allowed to react for two to five minutes at room temperature before being read spectrophotometrically at a wavelength of 414 nm. Serum samples were considered positive if the mean absorbance value was more than 1.5 times that of the mean negative control.

Evaluation of Stools and Definitions

All stools passed by the subjects were collected daily for the first two weeks. Then, 24-hour stool collections were carried out two days a week for two months. Stools were maintained on ice in plastic coolers in the subjects' homes and were transported each morning to the clinical research center, where a daily evaluation was performed and body temperature was recorded by the study nurses. The participants were instructed about the principles of hygiene and the importance of hand washing and were told that they could not have direct contact with young infants, pregnant women, or elderly or debilitated persons. They were asked to keep a daily diary detailing when stools were passed and symptoms occurred. They were given an oral electrolyte solution and told how to use it to treat diarrhea. Household contacts were given a description of the study, and the staff of the clinical research center monitored each household weekly for diarrheal illness. Details of diarrheal illness in the subjects or their contacts were obtained, and stool samples were collected and studied for conventional enteric pathogens when diarrhea was reported. All stools collected were examined for cryptosporidium, and oocysts were quantitated by direct immunofluorescence assay as described previously.²⁵

Stools were collected and categorized as formed (retaining their shape), soft (taking the shape of the container), or watery (pourable). Both soft and watery stools were considered unformed. To minimize the chance of detecting only passively excreted organisms given orally, cryptosporidium infection was defined as the excretion of oocysts in stool more than 36 hours after the ingestion of the gelatin capsule. Diarrheal illness was defined as the passage of three unformed stools in 8 hours or of more than three unformed stools in 24 hours in addition to the presence of one or more signs or symptoms of an enteric infection, including fever, nausea, vomiting, abdominal pain or cramps, and gas-related intestinal symptoms. Cryptosporidiosis was defined as infection (oocyst-positive stools) plus diarrheal illness as previously defined. The criteria for wellness employed in the study have been described elsewhere.²⁶

Statistical Analysis

To estimate the ID₅₀, linear-regression analysis was used to compare the percentage of infected subjects with the number receiving a specific dose (log transformed) of oocysts. To analyze the effect of the dose on the onset and duration of infection, the day the infection began and the number of days the infection lasted were plotted against the challenge dose, which was divided into three dose levels: 30 to 100, 300 to 500, and ≥1000 oocysts. The Kruskal-Wallis nonparametric analysis-of-variance test was used to compare the mean time to the onset of infection and the mean duration of infection for the various doses. The chi-square test was used to study the relation of the oocyst challenge to the occurrence of enteric symptoms.

RESULTS

The oocyst preparations examined were negative for retrovirus, enterovirus, myxovirus, aerobic and anaerobic bacteria, and animal virus-like or phage-like parti-

cles. The viability of the oocysts, as assessed by determining the excystation rate, was 84 percent both before and after round-trip shipment (Arizona to Texas to Arizona), indicating that the shipping conditions were not detrimental to the survival of the oocysts.

The intended and actual oocyst counts in each inoculum are presented in Table 1. In all cases the number of oocysts actually given to the subjects was close to the intended dose, with a coefficient of variation ranging from 3.4 to 20.1 percent. Table 2 lists important characteristics of each inoculum preparation. The age of the oocysts — measured from the time of initial collection to their administration to the subjects — ranged from 10 to 40 days. Within that range, there were minimal differences in the sporozoite yield, with values ranging from 43 to 52.7 percent. The respective percentage of viable oocysts according to 4',6-diamidino-2-phenylindole staining and the corresponding rate of excystation in vitro for four lots of oocysts were as follows: 63.5 percent and 84.4 percent, 82.3 percent and 79.0 percent, 83.7 percent and 85.0 percent, and 82.0 percent and 89.0 percent.

Of 112 potential study subjects, 19 (17 percent) were seropositive for anticryptosporidial antibody by ELISA. Of the 93 seronegative volunteers, 29 were selected for study and retested within two weeks after the ingestion of *C. parvum* oocysts to confirm antibody negativity. Twelve of the subjects were men and 17 were women. Three subjects were 20 to 25 years of age, 10 were 26 to 30, 6 were 31 to 35, 3 were 36 to 40, and 7 were 41 to 45. Six were black, 4 Hispanic, and 19 white.

The subjects ingested cryptosporidium in doses ranging from 30 to 1 million oocysts, and 18 (62 percent) had cryptosporidium infection (Table 3). The infections occurred at all dose levels. Linear-regression analysis of data presented in Table 3 yielded an r^2 of 0.983 and an ID_{50} of 132 oocysts. The ID_{50} of the Iowa strain of *C. parvum* in neonatal mice was 60 oocysts.

The excretion of oocysts was associated with the development of clinical enteric symptoms. One of 11 subjects who did not excrete oocysts (9 percent) had enteric symptoms on days 23 to 31 and passed a single soft stool 10 days after ingesting the oocysts. These symptoms were unrelated to the cryptosporidium challenge, and this subject was removed from further analysis. None of the other 10 uninfected subjects had enteric symptoms. In contrast, enteric symptoms developed in 11 of the 18 subjects (61 percent) who excreted oocysts. Seven of the 18 subjects (39 percent) also had diarrheal illness, thus meeting the criteria for clinical cryptosporidiosis. All seven subjects with clinical illness reported abdominal pain, cramps, and diarrhea; six had nausea; one reported gas-associated symptoms; and one reported vomiting. One had moderate dehydration and was given intravenous fluids. Selected clinical features of the illness in the seven subjects with induced cryptosporidiosis are presented in Table 4. The number of oocysts ingested by the subjects did not substantially influence the incubation period or the duration or severity of illness. The mean and median incu-

Table 1. Intended and Actual Numbers of *C. parvum* Oocysts Given Experimentally to the Subjects.

INTENDED CONCENTRATION	ACTUAL CONCENTRATION*	COEFFICIENT OF VARIATION (%)
30	34±3	9.6
100	108±22	20.1
300	313±24	7.6
500	504±19	3.8
1,000	1,129±160	14.2
10,000	11,460±1,048	9.1
100,000	113,904±3,926	3.4
1,000,000	1,139,040±39,256	3.4

*Values are the means ±SD of three or more counts.

bation periods for cryptosporidiosis were 9 and 6.5 days, respectively.

Four subjects who excreted oocysts but did not meet the criteria for diarrheal illness had enteric symptoms. Two received approximately 10,000 oocysts. One of the two had nausea, anorexia, vomiting, and abdominal pain on the second day after the challenge, with excretion of oocysts beginning five days later, but did not pass unformed stools. The second had nausea, headache, and abdominal pain on days 5 and 6 after the challenge and passed two watery stools. The third subject, who received approximately 1000 oocysts, reported anorexia and abdominal pain on days 31 and 32, during which time oocysts were excreted in formed stools. The fourth subject received approximately 500 oocysts and reported abdominal cramps on days 6, 7, and 9, when stools were positive for *C. parvum*. This subject passed two soft stools on day 7 and three on day 9.

Nonstatistically significant relations were identified between the size of the inoculum in the 18 infected subjects and the time to the onset of infection ($P=0.43$) and the duration of oocyst excretion ($P=0.06$) (Fig. 1). With the higher doses of oocysts, infection tended to occur sooner and last longer. There was a relation between the number of oocysts administered and the occurrence of one or more of the enteric symptoms. Eight of 13 subjects (62 percent) who received an intended

Table 2. Characteristics of the Inocula Used in the Challenge Studies.*

INOCULUM No.	DATE OF OOCYST PRODUCTION	DATE OF INOCULATION	AGE OF OOCYSTS	EXCYSTATION RATE†	SPOROZOITE YIELD‡
			days	%	%
1	2/9/93	3/12/93	31	89.9	51.0
2	3/16/93	3/26/93	10	84.4	49.0
3	5/12/93	5/27/93	15	79.0	50.0
4	5/12/93	6/17/93	36	ND	ND
5	6/17/93	7/13/93	26	85.0	50.0
6	8/5/93	9/14/93	40	88.0	43.0
7	1/18/94	2/3/94	16	89.0	52.7
8	1/18/94	2/24/94	37	88.5	ND

*ND denotes not done.

†The excystation rate is the number of shells/(number of intact cells + number of shells) × 100.

‡The sporozoite yield is the number of sporozoites/4 (number of intact oocysts + number of shells) × 100.

Table 3. Rate of Infection, Enteric Symptoms, and Clinical Cryptosporidiosis, According to the Intended Dose of Oocysts.*

INTENDED DOSE OF OOCYSTS	NO. OF SUBJECTS	INFECTION	ENTERIC SYMPTOMS		CRYPTO-SPORIDIOSIS
			number (percent)		
30	5	1 (20)	0	0	0
100	8	3 (37.5)	3 (37.5)	3 (37.5)	3 (37.5)
300	3	2 (66.7)	0	0	0
500	6	5 (83.3)	3 (50)	2 (33.3)	2 (33.3)
≥1000†	7	7 (100)	5 (71.4)	2 (28.6)	2 (28.6)
Total	29	18	11	7	7

*Linear regression analysis of the data yielded an r^2 of 0.983 and an ID_{50} of 132 oocysts.

†The intended dose was 1000 oocysts in two subjects, 10,000 in three, 100,000 in one, and 1 million in one.

dose of 500 or more oocysts were symptomatic, as compared with 3 of 16 (19 percent) of those receiving fewer than 500 oocysts (chi-square = 5.58, $P = 0.018$). No secondary spread was documented despite active surveillance of 30 household contacts.

DISCUSSION

The parasite *C. parvum* is an important emerging microbial threat to the general population and especially to high-risk groups.^{3-5,7,25,27} Limited serologic surveys suggest that cryptosporidiosis is common in virtually all communities. Serologic evidence of past infection is found in 15 percent or more of the U.S. population.^{16,27-29} However, almost 100 percent of people living in one tropical region of the developing world had serum anti-*C. parvum* antibodies,⁶ which suggests an association between the frequency of past infection and the general level of hygiene and sanitation. The seropositivity rate in our study is comparable to that in earlier studies among non-dairy-farming adults from Wisconsin (24 percent)²⁷ and U.S. Peace Corps volunteers (32 percent).²⁹

We established a safe model of human cryptosporidiosis in healthy adult volunteers given a single *C. parvum*

Table 4. Selected Clinical Features of Seven Subjects with Clinical Cryptosporidiosis.

SUBJECT NO.	INTENDED DOSE	INCUBATION PERIOD	DURATION OF ILLNESS*	MAXIMAL NO. OF UNFORMED STOOLS PER DAY	NO. OF UNFORMED STOOLS PER ILLNESS	TOTAL STOOL WEIGHT PER EPISODE OF DIARRHEA
				days	hr	
1	100	7	58	7	7	1.24
2	100	9	87	4	9	0.71
3	100	5	65	7	11	1.39
4	500	5	86	11	31	2.06
5	500	11	75	5	12	2.14
6	10,000	4	72	7	11	0.65
7	1,000,000	22	72	4	8	0.40
Mean		9	74	6.4	12.7	1.23

*The duration of diarrheal illness was calculated from the passage of the first unformed stool until the passage of the last unformed stool, after which the subject was deemed well.

strain. With the use of regression analysis, the ID_{50} for the Iowa strain of *C. parvum* in this population was calculated as 132 oocysts, a number comparable to that for *G. lamblia*.¹⁸ Interestingly, this ID_{50} is similar to that in neonatal mice (60 oocysts). In our study enteric symptoms developed in 11 of 18 subjects (61 percent) who excreted oocysts, and 7 (39 percent) were considered to have clinical cryptosporidiosis consisting of diarrhea and one or more associated symptoms, most of-

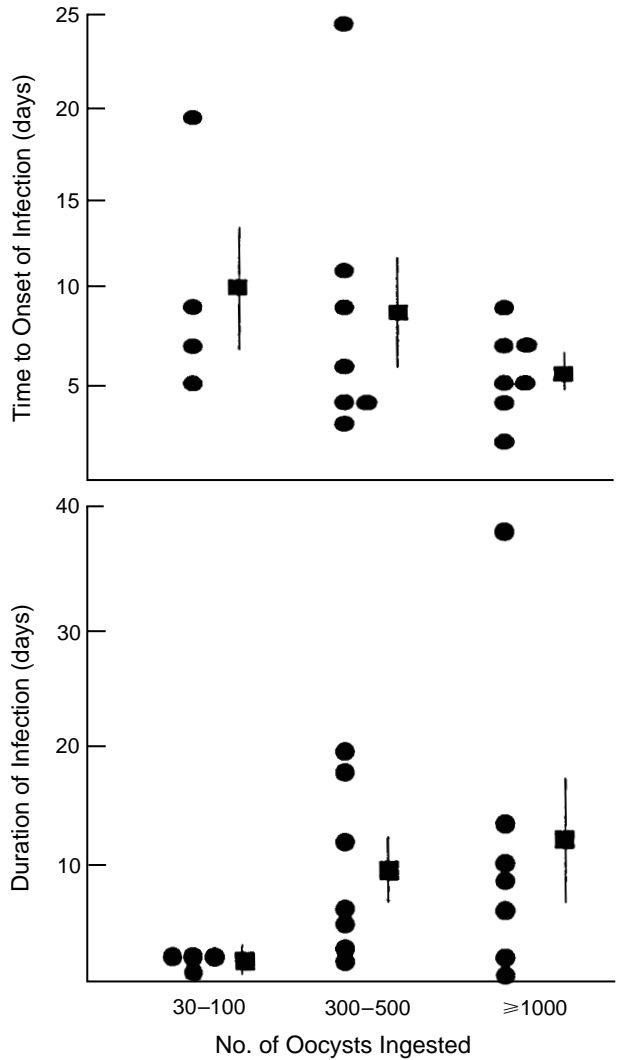


Figure 1. Relation between the Size of the Inoculum and the Onset and Duration of Infection.

Each circle represents a single infected subject. The means (squares) and standard errors (vertical lines) are indicated.

ten nausea, abdominal cramps, and pain. Although not statistically significant, our data suggest that the size of the inoculum influenced the time to and the duration of oocyst excretion but not the incubation period of illness or its severity. Statistical significance for these variables might have been reached if a larger number of subjects had been studied. With an average incubation period

of nine days, the illness was typically mild, consisting of the passage of 12.7 unformed stools over a period of three days. A previous study reported an average incubation period of 7 days (range, 1 to 12) and a mean duration of naturally occurring cryptosporidiosis of 12 days (range, 2 to 26).³⁰

The milder illness in our study may relate to a number of factors. The subjects were in excellent health, and serum samples from all subjects were negative for cryptosporidium antibody. Persons with base-line antibodies to the parasite may have increased susceptibility to the illness, as has been shown for Norwalk virus infection.³¹ Also, the virulence of *C. parvum* isolates may vary. Since we were not studying the natural history of experimental disease, all subjects with diarrheal illness were given five days of paromomycin.

These data on healthy volunteers will be further used to predict the likelihood of enteric infection after exposure to drinking water contaminated with low levels of cryptosporidium, as has been done for *G. lamblia*, entamoeba, and other organisms.^{19,20} This information should be useful for evaluating the adequacy of current drinking-water standards and the contribution of contaminated drinking water to the overall morbidity from cryptosporidiosis. Accurate extrapolation of our data on induced illness to the naturally occurring disease will require additional research on the quantitation of cryptosporidium oocysts in drinking-water sources and their viability and infectivity. Studies in volunteers are under way to determine the role of primary infection in inducing protective immunity. Previously exposed volunteers are being rechallenged with the same *C. parvum* strain one year after the initial study.

We are indebted to Salma Marani for data-management services; to John J. Mathewson, Ph.D., for performing bacteriologic studies; to Lillian M. Stark, Ph.D., Florida Department of Health and Rehabilitative Service, for performing the tissue-culture studies; to Fred P. Williams, Environmental Monitoring Systems Laboratory, for performing the electron-microscopical studies; to Melinda Cox, Nazario Siytangco-Johnson, Phu Nguyen, Janice Jackson, and Marilyn M. Marshall for providing laboratory support for the studies; and to Julie Kincaid, R.N., Terry Kubiak, R.N., Nai-hui Chiu, R.N., Paula Officer, R.N., Madelene Jewell, R.N., and Inge Weiser, R.N., for caring for the volunteers.

REFERENCES

- Moon HW, Woodmansee DB, Harp JA, Abel S, Ungar BLP. Lactal immunity to enteric cryptosporidiosis in mice: immune dams do not protect their suckling pups. *Infect Immun* 1988;56:649-53.
- Wolfson JS, Richter JM, Waldron MA, Weber DJ, McCarthy DM, Hopkins CC. Cryptosporidiosis in immunocompetent patients. *N Engl J Med* 1985;312:1278-82.
- Jokipii L, Pohjola S, Jokipii AMM. Cryptosporidiosis associated with traveling and giardiasis. *Gastroenterology* 1985;89:838-42.
- Current WL, Reese NC, Ernst JVD, Bailey WS, Heyman MB, Weinstein WM. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *N Engl J Med* 1983;308:1252-7.
- Tangermann RH, Gordon S, Wiesner P, Kreckman L. An outbreak of cryptosporidiosis in a day-care center in Georgia. *Am J Epidemiol* 1991;133:471-6.
- Newman RD, Lima AAM, Zu S-X, Guerrant RL, Wuhib T, Sears CL. Household epidemiology of *Cryptosporidium parvum* infection in an urban community in northeast Brazil. *Ann Intern Med* 1994;120:500-5.
- Lederberg J, Shope RE, Oaks SC Jr, eds. *Emerging infections: microbial threats to health in the United States*. Washington, D.C.: National Academy Press, 1992.
- Isaac-Renton JL, Fogel D, Stibbs HH, Ongerth JE. *Giardia* and *Cryptosporidium* in drinking water. *Lancet* 1987;1:973-4.
- Korich DG, Mead JR, Madore MS, Sinclair NA, Sterling CR. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Appl Environ Microbiol* 1990;56:1423-8.
- Madore MS, Rose JB, Gerba CP, Arrowood MJ, Sterling CR. Occurrence of *Cryptosporidium* oocysts in sewage effluents and selected surface waters. *J Parasitol* 1987;73:702-5.
- LeChevallier MW, Norton WD, Lee RG. *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. *Appl Environ Microbiol* 1991;57:2617-21.
- Angus KW, Sherwood D, Hutchison G, Campbell I. Evaluation of the effect of two aldehyde-based disinfectants on the infectivity of faecal cryptosporidia for mice. *Res Vet Sci* 1982;33:379-81.
- Campbell I, Tzipori AS, Hutchison G, Angus KW. Effect of disinfectants on survival of *Cryptosporidium* oocysts. *Vet Rec* 1982;111:414-5.
- Finch GR, Black EK, Gyurek L, Belosevic M. Ozone inactivation of *Cryptosporidium parvum* in demand-free phosphate buffer determined by *in vitro* excystation and animal infectivity. *Appl Environ Microbiol* 1993;59:4203-10.
- Hayes EB, Matte TD, O'Brien TR, et al. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *N Engl J Med* 1989;320:1372-6.
- D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. *Ann Intern Med* 1985;103:886-8.
- Mac Kenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med* 1994;331:161-7.
- Rendtorff RC. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *Am J Hyg* 1954;59:209-20.
- Regli S, Rose JB, Haas CN, Gerba CP. Modeling the risk for *Giardia* and viruses in drinking water. *J Am Water Works Assoc* 1991;83:76-84.
- Rose JB, Haas CN, Regli S. Risk assessment and control of waterborne giardiasis. *Am J Public Health* 1991;81:709-13.
- Arrowood MJ, Sterling CR. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. *J Parasitol* 1987;73:314-9.
- Woodmansee DB. Studies of *in vitro* excystation of *Cryptosporidium parvum* from calves. *J Protozool* 1987;34:398-402.
- Campbell AT, Robertson LJ, Smith HV. Viability of *Cryptosporidium parvum* oocysts: correlation of *in vitro* excystation with inclusion or exclusion of fluorogenic vital dyes. *Appl Environ Microbiol* 1992;58:3488-93.
- Ungar BLP, Soave R, Fayer R, Nash TE. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *Cryptosporidium* in immunocompetent and immunocompromised persons. *J Infect Dis* 1986;153:570-8.
- Goodgame RW, Genta RM, White AC, Chappell CL. Intensity of infection in AIDS-associated cryptosporidiosis. *J Infect Dis* 1993;167:704-9.
- DuPont HL, Cooperstock M, Corrado ML, Fekety R, Murray DM. Evaluation of new anti-infective drugs for the treatment of acute infectious diarrhea. *Clin Infect Dis* 1992;15:Suppl 1:S228-S235.
- Lengerich EJ, Addiss DG, Marx JJ, Ungar BLP, Juranek DD. Increased exposure to cryptosporidia among dairy farmers in Wisconsin. *J Infect Dis* 1993;167:1252-5.
- Kuhls TL, Mosier DA, Crawford DL, Griffis J. Seroprevalence of cryptosporidial antibodies during infancy, childhood, and adolescence. *Clin Infect Dis* 1994;18:731-5.
- Ungar BLP, Mulligan M, Nutman TB. Serologic evidence of *Cryptosporidium* infection in US volunteers before and during Peace Corps service in Africa. *Arch Intern Med* 1989;149:894-7.
- Jokipii L, Jokipii AMM. Timing of symptoms and oocyst excretion in human cryptosporidiosis. *N Engl J Med* 1986;315:1643-7.
- Blacklow NR, Cukor G, Bedigian MK, et al. Immune response and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay. *J Clin Microbiol* 1979;10:903-9.