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Selected Summaries

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The Infectivity of *Cryptosporidium parvum* in Healthy Volunteers. DuPont HL, Chapell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W.
N Engl J Med 1995;332:855–9.

Summary: Small numbers of *Cryptosporidium parvum* oocysts can remain behind and contaminate even treated drinking water, making it an important source of human infection. Ingestion of oocysts can cause diarrheal disease in normal as well as immunocompromised hosts, with a tendency to protracted diarrhea in the latter.

Aims, subjects, and methods: To determine the infectious dose of *C. parvum*, 29 healthy adults (students and administrative and research staff members of the Texas Medical Center), after being given ample information, volunteered to become infected with oocysts. Subjects were informed that the organism could 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. All participants were in good health and had normal immune status. Furthermore, they were required to not be pregnant and to be tuberculin-negative and negative for *C. parvum* on enzyme-linked immunosorbent assay (ELISA). The organism used was originally isolated from a calf in Iowa and subsequently propagated in 1-day-old calves at the University of Arizona. From their feces, oocysts were isolated, purified, stored, cooled, and shipped overnight to Texas.

Diverse culturing of inoculums was done to exclude the presence of viral, phagelike, and bacterial agents. Viability was tested comparing in vitro excystation in corresponding batches of inoculums, before and after shipment. Infectivity of oocysts was confirmed in laboratory mice with an initial dose of 60 oocysts per mouse, as previously described (*Appl Environ Microbiol* 1990;56: 1423–8). After careful assessment of the number of oocysts in each lot, dilutions were performed to obtain concentrations ranging from 30 to one million, which were placed in gelatin capsules.

Within 1 h of preparation, capsules and 250 ml of buffered saline were given to the subjects, while no food or drink was allowed for 90 min before and after ingestion of the capsule. The subjects' household contacts were monitored weekly. All stools passed were collected for 2 weeks and thereafter 2 days per week for 2 months. Details of diarrheal disease in the subjects and their contacts were obtained. In cases of diarrhea, stools were also examined for conventional pathogens apart from *C. parvum*. Cryptosporidiosis was defined as infection (oocyst-positive stools) plus diarrheal illness.

Results. Of 112 volunteers, 19 (17%) were seropositive for anticryptosporidial antibody by ELISA. Of the remaining 93 seronegative volunteers, 29 were finally selected for the study. Eighteen (62%) of them developed cryptosporidium infection, within the whole range (30 to one million) of dose levels. Linear regression analysis yielded a mean infective dose (ID_{50}) of 132 oocysts of *C. parvum*, compared with an ID_{50} of 60 oocysts of the same Iowa strain in neonatal mice. None of the 10 uninfected subjects (one being excluded from the study because of nonchallenge-related enteric symptoms) had symptoms of enteric infection. Of the 18 oocyst-excreting subjects, 11 (61%) did have symptoms, which included fever, nausea, vomiting, abdominal pain or cramps, and gas-related intestinal symptoms. Seven (39%) also had diarrheal illness and were thus considered to have clinical cryptosporidiosis.

Illness lasted 58–87 (mean 74) h, with a maximum number of four to 11 (mean 6.4) unformed stools per day. All seven reported abdominal pain and cramps, six had nausea, one reported gas-related symptoms, and one had vomiting. The number of ingested oocysts did not substantially influence the length of the incubation period (mean and median 9 and 6.5 days, respectively) nor the duration or severity of illness. However, with higher doses of oocysts, infection tended to occur sooner and last longer. Since the aim of the study was not to outline the natural history of experimental cryptosporidiosis, all subjects with diarrheal disease received a 5-day course of paromomycin. Of 30 household contacts, no secondary spread was documented.

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.

Comment: Since 1976, *C. parvum* has been recognized as a human pathogen, especially in the immunocompro-

mised host. It has been noted to be an important public health problem in the past decade. By now it is a well-established cause of acute diarrhea in different parts of the world. This study in volunteers confirms the high infectivity of *Cryptosporidium* organisms by means of its oocysts, which are known to be resistant to chlorination but are killed by high temperatures and freezing. Infection can be transmitted through person-to-person or animal-to-person contact, ingestion of fecally contaminated water or food, or contact with fecally contaminated environmental surfaces. In the U.S.A., 65–97% of surface waters contain *Cryptosporidium* oocysts, and cryptosporidiosis has become a notifiable disease since January 1995 (*MMWR* 1995;44/no.RR-6:1–16).

As demonstrated in the study, only a small number of viable oocysts is needed for infection. Illness in a healthy individual who has not been in previous contact with the parasite is acute, enteric, and self-limiting, lasting 3 days on average. However, even in immunocompetent children, chronic diarrhea due to proximal intestinal damage and followed by spontaneous recovery is known to occur (*Gut* 1992;33:1057–61). As in giardiasis, the worldwide prevalence of cryptosporidiosis is associated with low levels of hygiene and sanitation. For example, in Brazil, poor families have high transmission rates and seropositivity rates ≤100% (*Ann Intern Med* 1994;120:500–5). In poor Latin American children, multiple infections with intestinal protozoan and helminthic parasites are common and may contribute to the fact that, for example, among Brazil's impoverished families malnutrition prevalences in children have doubled between 1974 and 1986 (*Lancet* 1994;343:48).

For young children living in the Third World, inoculation with *C. parvum* is part of a parcel of gastrointestinal infections. In less-developed countries prevalences of cryptosporidiosis among those who are seeking medical care range from 0.5% to 32% (*Pediatr Infect Dis* 1986;5: 117–30; *J Trop Pediatr* 1993;39:132–6). A study from Guinea Bissau (West Africa) shows that cryptosporidiosis contributes to diarrheal mortality in healthy children <5 years old (*Br Med J* 1993;307:417–20). It is not clear whether seropositivity protects against reinfection or modifies illness. DuPont et al. are presently undertaking studies in healthy volunteers to determine the role of primary infection in inducing immunity. Like infection with *G. duodenalis*, an asymptomatic carrier state or a long-standing (intermittently) symptomatic state of *C. parvum* infection appears to occur in relatively healthy children (*Am J Trop Med Hyg* 1993;49:63–7). This finding could suggest that at a population level, similar to findings in giardiasis (*Am J Clin Nutr* 1986;43:395–405), a significant interaction between chronic *Cryptosporidium* infection and nutrition could play a role in the development of undernutrition. Studies are needed to investigate this issue.

In the presence of a compromised immunity, *C. parvum* infection has a clinically variable course, ranging from acute and dehydrating diarrhea to protracted diarrhea with a debilitating effect on nutritional status (*Gut* 1988;29:593–7). Generally, an effective immune system is required to overcome *Cryptosporidium* infection, and so far no drugs have been able to cure cryptosporidiosis in the immunocompromised host. Important questions remain unanswered: the biology of the parasite is little known, and therefore chemotherapy cannot be targeted. Furthermore, the role of parasite virulence versus preexisting mucosal B-cell immunity needs clarification (*Lancet* 1995;345:1128–9). Sufficient levels of specific secretory IgA antibodies to *Cryptosporidium* have been reported not to be able to control infection in AIDS patients (*J Med Microbiol* 1994;40:10–4). At present, with regard to *Cryptosporidium* and its host there are not many answers to be given.

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