CRYPTOSPORIDIUM HOMINIS: EXPERIMENTAL CHALLENGE OF HEALTHY ADULTS

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Abstract. Cryptosporidium hominis causes diarrhea in humans and has been associated with community outbreaks. This study describes the infectivity, illness, and serologic response after experimental challenge of 21 healthy adult volunteers with 10–500 C. hominis (TU502) oocysts. Sixteen subjects (76.2%) had evidence of infection; the 50% infectious dose (ID50) was estimated to be 10–83 oocysts using clinical and microbiologic definitions of infection, respectively. Diarrhea occurred in 40% of subjects receiving 10 oocysts with a stepwise increase to 75% in those receiving 500 oocysts. A serum IgG response was seen in those receiving more than 30 oocysts. Greatest responses were seen in volunteers with diarrhea and oocyst shedding. Volunteers with no evidence of infection had indeterminant or negative IgG responses. Cryptosporidium hominis is infectious for healthy adults (ID50 = 10 oocysts) and is clinically similar to C. parvum-induced illness. In contrast to C. parvum, C. hominis elicited a serum IgG response in most infected persons.

INTRODUCTION

Molecular techniques have shown that two major species, Cryptosporidium parvum and C. hominis, are responsible for most human cases of cryptosporidiosis.1,2 Cryptosporidium hominis infections are transmitted directly or indirectly from person to person, but have also been found on rare occasions in animals.3–5 In addition, C. hominis has been experimentally transmitted to ruminants, and sensitive polymerase chain reaction (PCR) tests have detected C. hominis subpopulations in humans and animals excreting C. parvum oocysts.6,7 In contrast, C. parvum is transmitted zoonotically and has been identified in multiple mammalian species, including cattle, horses, sheep, goats, and other domesticated and wild species.8

Serologic studies indicate that ≥25% of the U.S. population have been exposed to Cryptosporidium.9–18 The number is even higher in developing countries or in areas with poor sanitation and drinking water quality.11–15 Outbreaks of diarrhea from C. hominis or C. parvum are typically associated with contaminated recreational or drinking waters.16 Both Cryptosporidium species have caused community outbreaks of diarrhea, but urban populations are more often infected with C. hominis.17–19 Also, C. hominis appears to predominate in most studies of persons infected with human immunodeficiency virus.20,21 Over the past few years, experimental studies of C. parvum in healthy adults have contributed important information regarding infectivity (50% infectious dose [ID50]), natural history of the disease, and host immune response.22–26 Overall, the onset of diarrhea in the volunteers typically occurred between days 4 and 7 post-challenge and lasted for another 4–7 days. Oocyst shedding usually began on days 6–8 post-challenge, lasted for 3–8 days, and often continued for a few days after diarrhea resolved. In contrast, the infectious dose (ID50) among four isolates varied from 9 to 1,042 oocysts. In further studies, volunteers who had pre-existing serum antibody to Cryptosporidium antigens showed a relative resistance to re-infection when challenged with the homologous isolate (Iowa). In this population, ID50s were approximately 20-fold higher than in those subjects without pre-existing specific antibodies.

Since knowledge of C. hominis infections has been limited to case reports and outbreak situations, little information exists regarding C. hominis infectivity and illness, particularly in immunocompetent hosts. Furthermore, serologic response to C. hominis has not been previously studied. Thus, this experimental challenge with C. hominis oocysts is the first study to examine infectivity, illness, and the serum IgG response to homologous antigens in healthy individuals. These data are important not only to advance our understanding of C. hominis pathogenicity, but also to provide essential information for risk assessment and protection of the drinking water supply.

MATERIALS AND METHODS

Study population. The study population consisted of healthy adults of both sexes and all races (age range = 18–50 years) recruited by advertising in the Texas Medical Center and in Houston newspapers. This study was done following inclusion and exclusion criteria and study-related procedures as previously described.25,26 After informed consent was obtained, volunteers agreeing to participate in the study were initially serologically screened for the presence of antibodies to Cryptosporidium. Volunteers who were negative for specific IgG underwent a thorough medical examination to identify any abnormalities that might be present. The challenge study was carried out in the University Clinical Research Center at the Memorial-Hermann Hospital (Houston, TX). The study was reviewed and approved by The University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects. Informed consent was obtained from all subjects prior to the initiation of the study.

Serologic testing. Blood was collected prior to challenge and at days 5, 10, 30, and 45 post-oocyst challenge. Sera were separated by centrifugation, tested for pre-challenge antibodies, and stored at −80°C. Antibodies to Cryptosporidium were detected by enzyme-linked immunosorbent assay using dis-
rupted C. parvum or C. hominis oocysts as previously described. Cryptosporidium parvum antigens were used to assess pre-challenge antibodies to Cryptosporidium in volunteers. Known positive and known negative control sera were run on each microtiter plate. Positive sera were defined as those with a mean absorbance (414 nm) ≥ 1.5 times the negative control (mean optical density [OD] = 0.115).

Cryptosporidium hominis antigens were used to examine the IgG response at days 0, 5, 10, 30, and 45. In each case, negative and positive control sera (to C. parvum antigens) were included in each microtiter plate. All tests were done in duplicate. Negative and positive control sera yielded mean ODs of 0.143 and 0.262, respectively. For each subject, the mean absorbance at day 0 was subtracted from the peak post-challenge absorbance (day 30 or day 45) and expressed as the change in OD (Δ OD). The Δ OD was plotted for each volunteer, and three groupings were apparent. Lowest Δ ODs (0.0–0.054) were considered negative, mid-group Δ ODs (0.079–0.113) were considered indeterminate, and highest Δ ODs (≥ 0.147) were considered positive.

Oocyst propagation and isolation. Cryptosporidium hominis oocysts (TU502) were originally isolated from a child with cryptosporidiosis and were propagated in the gnotobiotic piglet model. Oocysts produced in this model were purified by the ether/Nycodenz method with a final purification step using the micro-scale cesium chloride gradient technique described by Widmer and others, and Arrowood and Donaldson. Purified oocysts were placed in 2.5% potassium dichromate for shipment to The University of Texas School of Public Health in Houston. Oocyst excystation and viability were determined within 48 hours of volunteer challenge. Excystation assays were carried out as previously described. Viability of oocysts was determined with a BacLight Viability Kit (Molecular Probes Inc., Eugene, OR) as directed by the manufacturer. Oocyst preparation and delivery to volunteers has been described elsewhere. Oocyst suspensions were subjected to serial dilution with phosphate-buffered saline, and replicate hemacytometer counts (n ≥ 6) were done to estimate the number of oocysts per unit volume. Once the desired concentration was reached, a 10-μL aliquot of the suspended oocysts was removed and instilled into gelatin powder contained in a capsule. The capsules were delivered to volunteers and ingested within one hour of preparation.

TU502 oocysts isolated from the original source as well as those passed in the gnotobiotic pig were genotyped using a restriction fragment length polymorphism located in the oocyst wall protein gene (COWP) as previously described. Subsequent passages in the gnotobiotic pig and stool samples from challenged volunteers were also tested in the same fashion. In addition, oocyst samples were genotyped with the species-specific PCR marker Lib13 and the Cp492 and Cp358 microsatellite markers.

Monitoring of volunteers. Volunteers were monitored as described. Briefly, each volunteer was examined daily for the first 14 days after challenge. A personal diary, which documented the number and time of stool passage and any gastrointestinal symptoms that may have occurred, was kept by each participant and audited daily by the nursing staff. Also, stool samples collected during the previous 24 hours were delivered to the laboratory for analysis. After the first two weeks of the study, volunteers were examined as above three times per week for four additional weeks and asked to provide at least two 24-hour stool samples per week.

Detection of oocyst shedding. Fecal specimens were collected from volunteers throughout the six-week study period and held at 4°C for no more than 24 hours prior to transfer to the laboratory. Specimens were then tested in duplicate for the presence of oocysts using a commercially available enzyme immunoassay (EIA) kit as described by the manufacturer (Prospect® Cryptosporidium microplate assay; Alexon-Trend, Ramsey MN). All specimens that were positive by EIA were quantified by immunofluorescent assay (IFA) (Merifluor C/G; Meridian Bioscience, Inc., Cincinnati, OH) as previously described.

Definitions of infection and illness. Illness attack rate was defined as the number of cases of diarrhea divided by the number of volunteers who were exposed. Infection was confirmed when fecal oocysts were detected by EIA, IFA, or both at ≥ 36 hours post-challenge. Criteria for diarrhea included passage of ≥ 200 g of unformed stool per day, ≥ 3 unformed stools in eight hours, or ≥ 4 unformed stool in 24 hours. Symptoms included ≥ 2 concurrent gastrointestinal complaints (such as abdominal pain/cramps, tenesmus, gas, nausea, vomiting, fecal urgency, or fecal incontinence) in the context of at least one unformed stool. Duration of diarrhea was measured as previously described. Cryptosporidiosis was defined as diarrhea in addition to ≥ 1 gastrointestinal symptoms with or without demonstrated oocysts within 30 days post-challenge.

Analysis of data. The oocyst dose sufficient to infect 50% of susceptible persons (ID50) was estimated using the cumulative endpoint method. Kruskal-Wallis analysis of variance (ANOVA) with Dunn’s multiple comparison tests were used to compare the IgG response (Δ OD) among clinical outcome groups. Analysis of variance with Welch’s correction was used to compare outcome values for onset, duration, and severity of illness between C. hominis and C. parvum isolates. A P value < 0.05 was considered statistically significant. Data were analyzed using Instat software (GraphPad Software Inc., San Diego, CA).

RESULTS

Description of study population. Fifteen women (71.4%) and six (28.6%) men ranging in age from 19 to 50 years (mean = 32.9 years, median = 34 years) were enrolled in the study. Ethnicity of the volunteers was 12 Black (57.1%), 8 White (38.1%), and 1 (4.8%) Hispanic.

Stability and delivery of C. hominis oocysts. Five batches of TU502 were produced in gnotobiotic pigs and used in volunteer challenge studies. All challenge doses were given within six weeks of oocyst production. At the time of volunteer challenge, oocyst excystation rates and viability ranged from 67–83% and 66–87%, respectively. Intended (actual ± SD) doses were 10 (10.3 ± 5.1), 30 (32.6 ± 6.5), 100 (105.3 ± 13.5), and 500 oocysts (500.8 ± 34.8). The coefficient of variations in the doses were 49.5%, 19.6%, 12.8%, and 6.9%, respectively.

Genotypic analysis. TU502 oocysts passaged in pigs and oocysts excreted by four volunteers were genotyped using one restriction fragment length polymorphism marker (COWP), one species-specific PCR assay (Lib13), and two microsatellite length polymorphisms (Cp492 and Cp358) (Figure 1). These analyses confirmed that volunteers were excreting C.
**hominis** oocysts and showed no changes in the parasite population after pig-to-human passage. The Lib13 amplicons were obtained using the *C. hominis*-specific primers for three of four volunteer samples (Figure 1, top). Control *C. parvum* and *C. hominis* samples were tested in parallel and the expected negative and positive amplification, respectively, was observed. The same Cp358 amplicon was amplified from four pigs and three human samples, but this marker was less informative because the same allele was also found in two *C. parvum* controls. The Cp492 amplicon was amplified from three volunteer and four pig samples and displayed the same allele diagnostic for *C. hominis*, which was different from that obtained from the *C. parvum* control isolate Moredun (MD) (Figure 1, bottom). One volunteer sample failed to amplify with Lib13, Cp358, and Cp492.

**Infectivity of *C. hominis* oocysts.** Only those persons who were IgG negative for antibodies to *Cryptosporidium* were enrolled in the study. Of the 21 volunteers who received a challenge dose, 13 developed diarrhea, and 9 had oocysts detected in fecal samples (Table 1). All nine oocyst-positive subjects had diarrhea and/or additional gastrointestinal symptoms. In contrast, 7 of 13 subjects with diarrhea and/or GI symptoms had no detectable oocysts. Five other volunteers had no evidence of infection. None of the volunteers who experienced a diarrhea had evidence of other enteric pathogens, despite having a complete microbiologic workup of all diarrheic stools.

Dose-response curves were calculated using two different outcome variables: oocyst shedding with or without diarrhea (microbiologic definition) or diarrhea with or without detectable oocysts (clinical definition) (Figure 2). For oocyst-positive persons (*n* = 9), the estimated ID<sub>50</sub> was 83 oocysts compared with 10 oocysts using the clinical definition.

**Clinical outcomes of *C. hominis* infections.** Diarrhea developed in 13 volunteers who ingested TU502 oocysts, yielding an illness attack rate of 61.9%. Three volunteers who did not meet criteria for diarrhea reported at least one unformed stool along with two or more additional gastrointestinal symptoms (Table 1). Six (66.7%) of the nine volunteers who had oocysts detected in their stools developed diarrhea. All volunteers received oral rehydration therapy when they developed diarrhea. One volunteer (no. 134) presented to the Clinical Research Center with mild dehydration, was rehydrated with intravenous fluids, and released after 15 hours of overnight observation.

A dose-response relationship was seen in subjects that developed diarrhea. Those receiving lower doses were less likely to experience a diarrheal illness. The doses and percent of

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* Positive (+), negative (−), and indeterminant (I) outcomes are indicated. See Materials and Methods for definitions of each category.
volunteers with diarrhea were as follows: 10, 40%; 30, 60%; 100, 71.4%; and 500, 75%. Interestingly and in contrast to previous studies done with C. parvum, asymptomatic shedding was not seen in any of the volunteers receiving C. hominis oocysts.

The incubation period for diarrhea ranged from 2 to 10 days after oocyst challenge with a mean ± SD and median of 5.4 ± 2.7 days and 4 days post-challenge, respectively (Table 2). The duration of diarrhea also varied approximately 10-fold with a range of 49 hours (2 days) to 518 hours (21.6 days). Mean ± SD and median duration were 137.3 ± 142.3 (5.7 days) and 75 hours (3.1 days), respectively. Of note, however, three subjects experienced a symptomatic episode of 9, 13, or 21 days.

Severity of illness was evaluated by the number of unformed stools and the total unformed stool weight per diarrheal episode. The mean ± SD number of unformed stools was 8.9 ± 5.0 (median = 9), and the total stool weight was 1.08 ± 0.72 (median = 0.86) kilograms. The mean ± SD total number of unformed stools passed per day was 3.2 ± 1.0 and did not exceed five stools on any day.

Infection and illness parameters from volunteers challenged with C. hominis were compared with similar experiments using four C. parvum isolates (Iowa, UCP, TAMU, and MD, ANOVA with Welch’s correction). Onset, duration, or severity of diarrheal illness were not statistically different (\( P > 0.05 \)) among isolates.

**Post-challenge serum IgG response.** For each volunteer, the specific serum IgG response was assessed after challenge and compared with the pre-challenge value. Overall, baseline absorbance values had a mean ± SD of 0.183 ± 0.055. The kinetics of the response varied somewhat but all positive sera reached peak values by days 30 or 45. The response category (i.e., positive, indeterminant, or negative) for each volunteer was then grouped according to challenge dose (Table 3). None of the five volunteers receiving a challenge dose of 10 oocysts mounted a measurable serum IgG response despite the fact that all met the clinical definition of infection and four were microbiologically confirmed. Sixteen volunteers received higher oocyst doses: eight (50%) had a positive IgG response (all with diarrhea), five (45.5%) were indeterminant (three with no evidence of infection), and three (27.3%) were serum IgG negative (two with no evidence of infection).

Furthermore, the ΔOD for each volunteer was plotted against the clinical outcome (Figure 3). Since none of the five persons who were challenged with 10 oocysts showed an IgG response, these volunteers were not included in the analysis. Volunteers receiving ≥ 30 oocysts and who were asymptomatic with no detectable oocysts showed the lowest ΔODs (mean ± SD = 0.056 ± 0.049). Those with diarrhea but no detectable oocysts were slightly more reactive with a mean ± SD Δ OD of 0.148 ± 0.118. The highest values were seen in volunteers who had a diarrheal illness and detectable oocysts (0.220 ± 0.076). This latter category was significantly different in mean OD (\( P = 0.014 \)) than the no diarrhea, no oocysts category.

**DISCUSSION**

This study is the first report of experimental challenge with C. hominis (TU502) in healthy adult volunteers. The estimated ID\(_{50}\) for TU502 was 10–83 oocysts, depending on whether the microbiologic or clinical definition of infection

![Figure 2](image-url)
was used. In this study, consecutive stool samples from subjects with confirmed TU502 infections were not always positive, suggesting that the intensity of oocyst shedding was variable and sometimes below the detectable limit even on some days when diarrhea was present. Furthermore, although 13 of the volunteers developed a diarrheal illness characteristic of cryptosporidiosis, seven had no detectable fecal oocysts in any stool sample, suggesting that these persons had light infections that remained below the detectable limit. This phenomenon is comparable to several subjects in the *C. parvum* challenge studies.26 In those earlier studies where cryptosporidiosis could not be confirmed by IFA or EIA, additional testing using a more sensitive flow cytometric method (detection limit = 10^3/mL) showed that a low level of oocysts were present in IFA-negative, diarrheic stools (Chappell CL and others, unpublished data).

To address the possibility of diarrhea from other causes, all diarrheic stools were subjected to a complete microbiologic work-up, and no pathogens other than *Cryptosporidium* were detected. Furthermore, symptoms exhibited by volunteers did not include nausea and vomiting, as may be expected with viral gastroenteritis.

Finally, the high degree of infectivity (i.e., low ID_{50}) associated with TU502 is comparable to the most infectious *C. parvum* isolates (TAMU and Iowa) used in other volunteer studies. Since only one *C. hominis* isolate was tested, the potential variability in infectivity among isolates remains known. However, if *C. hominis* mimics *C. parvum*, significant phenotypic variability may be expected. This would be consistent with a similar level of genetic heterogeneity observed in a geographically diverse collection of *C. parvum* and *C. hominis* isolates.36 However, the occurrence and extent of this variation requires additional study. A recently described rodent model for *C. hominis* will enable a comparative study of genetically distinct *C. hominis* isolates.37 To our knowledge, the *C. hominis* isolate used in the volunteer studies, is the only laboratory-maintained *C. hominis* isolate. This isolate was also selected for a recently completed genome sequencing project.38

A dose-response relationship for diarrhea was evident in volunteers who were challenged with *C. hominis* oocysts. This was not observed in previous studies with *C. parvum* oocysts.22–24 Of note, however, an earlier study showed that enteric symptoms (including but not limited to diarrhea) were significantly (P = 0.018) more common in volunteers receiving higher oocyst doses (≥500) of the Iowa isolate.22

*Cryptosporidium hominis* circulates in human populations and has not been associated with zoonotic transmission. However, the gnotobiotic pig is susceptible to *C. hominis* infection and was especially useful for these studies given the relative ease of oocyst purification in the absence of bacterial flora in the animal’s gut. Although fewer oocysts were produced in comparison to *C. parvum* in calves, the number of purified oocysts derived from the gnotobiotic pig was sufficient for the described studies. We did, however, note important differences in the stability of the purified oocysts in storage. In past studies, *C. parvum* oocysts were kept in 2.5% potassium dichromate for at least three months without exhibiting significant loss of viability. In those studies, it was a simple matter to maintain a viability ≥80% prior to volunteer challenge. In contrast, *C. hominis* oocysts were less stable at room temperature, showing an accelerated decrease in oocyst survival compared with *C. parvum* oocysts, an observation consistent with earlier findings.32 Therefore, excystation rates of oocysts delivered to volunteers were between 67% and 80% at the time of volunteer challenge depending on the oocyst batch. The infectivity estimates reported herein do not include any corrections for the lower excystation rate, and thus the reported ID_{50} may be slightly overestimated.

Little is known regarding the initiation of the serologic response to *Cryptosporidium* and whether the predominant antigenic stimulus is delivered with the challenge dose, during the replication process, or both. Previous studies with a *C. parvum* isolate (Iowa) indicated that subjects failed to mount a serum IgG response after primary challenge, but 33% did after rechallenge one year later.39 Furthermore, oocyst challenge in volunteers with pre-existing specific serum IgG resulted in an anamnestic response.35 In contrast to *C. parvum* (Iowa isolate), *C. hominis* resulted in a serologic response in 8 (38.1%) of 21 challenged volunteers and in 8 (53.3%) of 15 who had evidence of infection. Interestingly, only volunteers receiving ≥30 oocysts had a serum IgG response, even though all had diarrhea or fecal oocyst shedding. Furthermore, the degree of response was influenced by post-challenge outcome. Volunteers who had a diarrheal illness and who shed detectable levels of oocysts yielded the highest responses. Thus, data from this study suggest that the *Cryptosporidium hominis* isolate is associated with a diarrheal illness in healthy adults.

### Table 3

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* Responses were categorized as the following changes (day 0 versus peak) in optical density over the six-week study period: negative, ≤0.054; indeterminant, 0.079–0.113; positive, ≥0.147.
† Volunteer had no diarrhea, gastrointestinal (GI) symptoms, or detectable oocysts.
‡ Volunteer had diarrhea and GI symptoms, but no detectable oocysts.

![Figure 3](image_url)

**Figure 3.** Serum IgG response to the homologous antigen in volunteers challenged with 30–500 *Cryptosporidium hominis* oocytes. Each point represents the change in optical density (OD) between day 0 and the peak day of response during a six-week period. *P* = 0.014, by Kruskal-Wallis analysis of variance.
otosporidium species used in the challenge as well as the challenge dose and post-challenge events are important contributors to the overall serum IgG response. These observations, however, need to be confirmed with a larger population of subjects and with other C. hominis isolates before broad generalizations can be made.

In summary, C. hominis oocysts are capable of causing infection and illness in healthy adults similar to that seen with C. parvum. The ID₅₀ of TU502 is in the low range compared with that of C. parvum isolates. However, it is unclear whether the TU502 isolate is representative of C. hominis isolates circulating in human populations. The data generated from the C. hominis dose response studies adds to the growing body of data regarding Cryptosporidium infectivity in immunocompetent humans and provides important and valuable infectivity estimates for use in risk assessment and the setting of water quality standards.⁴⁰

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