Host Cell Tropism Underlies Species Restriction of Human and Bovine *Cryptosporidium parvum* Genotypes

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It has been recognized recently that human cryptosporidiosis is usually caused by *Cryptosporidium parvum* genotype I ("human" *C. parvum*), which is not found in animals. Compared to *C. parvum* genotype II, little is known of the biology of invasion of the human-restricted *C. parvum* genotype I. The aims of the present study were (i) to explore and compare with genotype II the pathogenesis of *C. parvum* genotype I infection by using an established in vitro model of infection and (ii) to examine the possibility that host-specific cell tropism determines species restriction among *C. parvum* genotypes by using a novel ex vivo small intestinal primary cell model of infection. Oocysts of *C. parvum* genotypes I and II were used to infect HCT-8 cells and primary intestinal epithelial cells in vitro. Primary cells were harvested from human endoscopic small-bowel biopsies and from bovine duodenum postmortem. *C. parvum* genotype I infected HCT-8 cells with lower efficiency than *C. parvum* genotype II. Actin colocalization at the host parasite interface and reduction in levels of invasion after treatment with microfilament inhibitors (cytochalasin B and cytochalasin D) were observed for both genotypes. *C. parvum* genotype II invaded primary intestinal epithelial cells, regardless of the species of origin. In contrast, *C. parvum* genotype I invaded only human small-bowel cells. The pathogenesis of *C. parvum* genotype I differs from *C. parvum* genotype II. *C. parvum* genotype I does not enter primary bovine intestinal cells, suggesting that the species restriction of this genotype is due to host tissue tropism of the infecting isolate.

Cryptosporidium parvum, an intracellular parasite belonging to the phylum Apicomplexa, is an important cause of diarrheal disease worldwide (10, 13, 17). Although many *C. parvum* infections are self-limiting, severe manifestations may occur in immunologically incompetent and debilitated individuals (10, 16, 17). In the immunodeficient host, in particular those with AIDS, infection may be life-threatening, and the development of effective antimicrobial therapy for human cryptosporidiosis is only beginning to evolve (35, 44, 45).

To develop novel, effective treatments against cryptosporidiosis, a detailed understanding of the pathogenesis of the causative organisms is necessary. Traditionally, cryptosporidiosis in humans has been viewed as a zoonotic infection, with cattle being the principal environmental reservoir. However, recently it has emerged that humans are commonly infected with a *C. parvum* genotype (genotype I) only very rarely found in other species (45). Although *C. parvum* genotype I is restricted to humans, *C. parvum* genotype II can infect humans, cattle, and a wide variety of other animals (2, 33, 37, 40). A compelling case now exists for the redesignation of this subtype as a novel cryptosporidial species (*Cryptosporidium hominis* sp. nov.) based on molecular differences at several genetic loci and

in terms of oocyst shedding, disease severity, and site of infection (28, 34). For clarity, we continue to use here the terminology "genotype I" and "genotype II."

In most human epidemiological studies *C. parvum* genotype I isolates are identified in human stool samples more frequently than genotype II (21, 24, 25, 43, 47). However, understanding of *Cryptosporidium* pathogenesis to date has relied on studies with *C. parvum* genotype II isolates or isolates of unknown genotype. The emergence of a widely prevalent non-zoonotic *C. parvum* genotype has stimulated a major reassessment of our understanding of this disease in humans. In particular, the possibility that the mechanisms of transmission and pathogenesis of this organism differ from those associated with *C. parvum* genotype II has major implications for strategies aimed at controlling and treating human cryptosporidiosis. We have used here a combination of established in vitro tissue culture models and an ex vivo primary intestinal cell model of cryptosporidial infection to show that *C. parvum* genotype I differs in the pattern and severity of infection compared to *C. parvum* genotype II isolates. In addition, we show that bovine primary cells cannot be infected with *C. parvum* genotype I isolates, indicating that host cell tropism underlies the species restriction of this *C. parvum* subtype in vivo.

MATERIALS AND METHODS

C. parvum oocysts. *C. parvum* genotype II oocysts (Iowa strain) were obtained from a commercial source (Pleasant Hill Farms, Troy, Idaho). This genotype II

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strain was originally isolated from a calf by Harley Moon. It has been passaged through calves and purified from the fecal material by ether extraction, followed by a one-step sucrose gradient.

C. parvum genotype I isolates, 5942 and TU502, were isolated and genotyped at the *Cryptosporidium* Reference Laboratory and the Tufts University School of Veterinary Medicine, respectively. For the *C. parvum* 5942 isolate oocysts were purified from *Cryptosporidium*-positive feces collected by the *Cryptosporidium* Reference Unit at the Singleton Hospital as part of the National Collection of *Cryptosporidium* Oocysts. Fecal isolates were confirmed by microscopic inspection of modified Ziehl-Neelsen-stained smears (3) and PCR-restriction fragment length polymorphism analysis of the *Cryptosporidium* oocyst wall protein gene (38). Briefly, oocysts were separated by flotation from fecal debris by using a saturated sodium chloride solution and centrifugation for 8 min at $1,600 \times g$ (36). The floating material containing the oocysts was washed with deionized oocyst-free water, the oocysts were resuspended in deionized, oocyst-free water and stored at 4°C. To extract DNA, 200 μ l oocyst suspension was incubated at 100°C for 60 min, and DNA was extracted by using proteinase K digestion in lysis buffer at 56°C by using a spin-column filtration technique (QiAMP DNA minikit; Qiagen). DNA extracts were stored at -20°C prior to use. The primers cry-15 and cry-9 were used to amplify a 550-bp region of the *Cryptosporidium* oocyst wall protein gene, which was then subjected to restriction endonuclease digestion by *Rsa*I. The digestion products were separated by agarose (3% [wt/vol]) gel electrophoresis, visualized by using ethidium bromide (0.1 mg/100 ml), and recorded with a digital camera and KDS1D analysis software (Kodak). Product sizes were confirmed by comparison with a DNA molecular weight standard marker (Life Technologies). Oocyst suspensions confirmed as containing *C. parvum* genotype I isolates were resuspended in phosphate-buffered saline (PBS), and those containing sufficient oocysts after visual inspection of a sample by bright-field microscopy were used for infection assays. *C. parvum* TU502 is a well-characterized genotype I isolate that has been passaged through gnotobiotic piglets (1). Before host cells were infected, oocysts were decontaminated by an established technique (22). Briefly, they were washed twice with distilled water and then incubated with freshly prepared 10% (vol/vol) Chlorox bleach (Sigma-Aldrich) for 10 min on ice. After two further washes with ice-cold distilled water and once with RPMI 1640 (Bio-Whittaker) oocysts were resuspended at a concentration of 2×10^5 oocysts/ml and then used to infect cell monolayers.

Preparation of HCT-8 cells. Human ileocecal adenocarcinoma cells (HCT-8; ATCC CCL 244) were obtained from the American Type Culture Collection. Cells were maintained in 75-cm² tissue culture flasks in RPMI 1640 and 10% (vol/vol) fetal bovine serum (FBS; Sigma). The cells were grown as adherent monolayers in a 37°C and 5% CO₂ -95% humidified incubator. At 24 h prior to infection, the monolayers were treated with trypsin-EDTA (Bio-Whittaker) for 15 min at 37°C. The cells were grown on 25-mm Thermanox coverslips in six-well Costar tissue culture plates (Gibco) to 80 to 85% confluence.

Isolation of epithelial cells from human and bovine gastrointestinal cells. Epithelial cells from human and bovine duodenal biopsies were isolated as described previously (9). Human small bowel biopsy tissue was obtained from children undergoing endoscopy at Our Lady's Hospital for Sick Children after parental consent was obtained. Ethical approval for the present study was obtained from the Ethics Committee at the hospital. The biopsy tissue was placed in RPMI 1640 medium containing 10% (vol/vol) FBS and transported directly to the laboratory. Bovine small duodenal tissue was removed from cattle younger than 30 months immediately after slaughter at a local abattoir. Small specimens were taken from the tissue by using a biopsy forceps; placed in RPMI 1640 containing 10% (vol/vol) FBS, 100 μ g of streptomycin/ml, 100 U of penicillin/ml, and 2.5 μ g of amphotericin B (Sigma)/ml; and transported on ice to the laboratory. Cells from both tissues were then isolated by using similar procedures. The tissue was placed in Hanks balanced salt solution (Bio-Whittaker) without Ca²⁺ or Mg²⁺ containing 0.1 mM EDTA (Sigma) and 0.1 mM dithiothreitol (Sigma) and incubated at 37°C for 10 min with vigorous shaking. The supernatant was removed, and the tissue was placed in RPMI 1640 containing 0.05% (wt/vol) collagenase (Sigma) and incubated at 37°C for 15 min with vigorous shaking. After this procedure, the supernatant consisted of intestinal crypts and single cells. The supernatant was removed with a Pasteur pipette, and the cells and crypts were pelleted by centrifugation at $800 \times g$ for 5 min and then resuspended in RPMI 1640 medium containing 10% (vol/vol) FBS. Fresh collagenase solution was added to the biopsy tissue, and the procedure repeated three to four times until no more cells could be isolated. The isolated cells and crypts were kept on ice until 500 μ l was plated in 24-well Costar culture plates on 13-mm plastic coverslips with Dulbecco modified Eagle medium-Ham F-12 plus 10% (vol/vol) FBS, 8 μ g of insulin/ml, 10 μ g of gentamicin/ml, 50 μ g of hydrocortisone/ml, 100 μ g of streptomycin/ml, 100 U of penicillin/ml, and 2.5 μ g of amphotericin B/ml.

Cytokeratin staining of epithelial cells. To confirm that the isolated primary cells were epithelial in origin, the cells were stained for cytokeratin. Monolayers were fixed with 4% formaldehyde in PBS for 30 min. Cells were permeabilized with 1% (vol/vol) Triton X-100 in PBS for 10 min and were blocked with 0.5% (wt/vol) bovine serum albumin (BSA) for another 10 min. Monolayers were then incubated with monoclonal anti-PAN cytokeratin (Sigma) in 0.5% (wt/vol) BSA for 30 min, followed by goat anti-mouse fluorescein isothiocyanate (FITC; Dako) for another 30 min. The coverslips were mounted by using fluorescence mounting medium (Dako) and examined with a fluorescence microscope. Control cells were stained as described above, but the primary antibody was omitted.

Infection assay. The maintenance medium was removed and 2×10^5 *Cryptosporidium* oocysts in 2 ml of growth medium were added to the cell monolayers. The multiplicity of infection was approximately five oocysts/cell. The plates were placed at 37°C in a 5% CO₂-95% air humidified incubator. After incubation for 3 h the infected monolayers were washed with PBS to remove unexcysted oocysts, empty oocyst walls, and toxic materials that may have been liberated from the oocysts. Then, 2 ml of growth medium containing 100 U of penicillin/ml and 100 μ g of streptomycin/ml was added. Primary cells were infected for up to 24 h, and HCT-8 cells were infected for up to 72 h.

Lectin VVL staining. Parasites were detected in the monolayers by using Lectin VVL (a plant lectin from *Vicia villosa*) staining, as previously described (18). Monolayers were fixed with 4% formaldehyde in PBS for 30 min, permeabilized with 1% Triton X-100 in PBS for 10 min, and blocked with 0.5% (wt/vol) BSA for an additional 10 min. The monolayers were then incubated with 1 μ g of conjugated lectin VVL-biotin (B1235; Vector Laboratories)/ml in 0.5% (wt/vol) BSA, followed by 1 μ g of streptavidin-CY3 (Sigma)/ml. The coverslips were mounted by using fluorescence mounting medium (Dako) and then examined by fluorescence microscopy with a $\times 50$ water immersion lens. The infected cells in 10 high-power fields were counted, and the percent rate of infection (number of infected cells/total number of cells $\times$ 100) was calculated. All experiments were performed in triplicate and expressed as the mean percent cells infected $\pm$ the standard deviation of the mean. Uninfected monolayers were used as a control.

Host cell actin was stained by using phalloidin conjugated to FITC. We used 5 μ g of FITC-phalloidin (Sigma)/ml, together with 1 μ g of Lectin VVL/ml, for 30 min. Slides were examined by using a laser scanning confocal microscope (Bio-Rad MRC 1024).

Effect of cytoskeletal inhibitors on *C. parvum* invasion of gastrointestinal cells. To investigate the importance of cytoskeletal components during invasion, the actin-disrupting drugs cytochalasin D and cytochalasin B (Sigma) and microtubule depolymerizing drugs colchicine and vincristine (Sigma) were used. Stock solutions of the four drugs were prepared and diluted to their final concentration by using culture medium. Three separate experiments were performed. In the first set of experiments, HCT-8 cells and primary cells were incubated for 1 h in an assay medium containing either 1 μ g of cytochalasin D/ml, 10 μ g of cytochalasin B/ml (7), or a range of concentrations of colchicine and vincristine (48) for 1 h at 37°C. The treated monolayers were washed twice with PBS and infected with 2×10^5 *Cryptosporidium* oocysts/ml for either 48 h (HCT-8 cells) or 22 h (primary cells). In a second set of experiments cell monolayers were infected as described above but without removing the cytoskeletal inhibitor during the infection assay. Finally, in order to assess the effect of cytoskeletal inhibition on the parasite, oocysts were incubated with a range of concentrations of either colchicine, vincristine, cytochalasin D, or cytochalasin B for 15 min at 37°C. Oocysts were then washed twice with PBS before being used to infect monolayers. Untreated monolayers or oocysts were used as a control.

Statistical analysis. All results were expressed as the mean percentage of cells infected $\pm$ the standard deviation of triplicate experiments. Means were compared by using the nonparametric Mann-Whitney U test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Invasion of HCT-8 cells by *Cryptosporidium* isolates. The patterns and degrees of invasiveness of *C. parvum* genotypes I and II were compared. Both genotypes invaded HCT-8 cells. However, genotype I entered HCT-8 cells with significantly lower efficiency than did genotype II. Specifically, $55.8\% \pm 1.01\%$ of cells were infected by genotype I, and $88.9\% \pm 1.54\%$ of cells were infected with genotype II (*P* = 0.002). The invasion efficiency of HCT-8 cells by both isolates of genotype I was similar. The pattern of infection differed according to the or-

FIG. 1. Comparison of the pattern of infection of *C. parvum* genotypes I and II in HCT-8 cells. Genotype I *C. parvum* invasion occurred in clusters (A), whereas genotype II *C. parvum* invasion was evenly distributed throughout the monolayer (B). Slides were examined by using a fluorescence microscope. Magnification, $\times 400$.

igin of the isolates. Whereas *C. parvum* genotype II infected HCT-8 cells evenly (Fig. 1B), internalized genotype I parasites formed discrete clusters throughout the cell monolayers (Fig. 1A).

Host cell actin cytoskeletal rearrangement is similar after invasion of HCT-8 cells by both genotype I and genotype II *Cryptosporidium* isolates. The effects on the host cell actin cytoskeleton after invasion with both genotypes were compared by using confocal scanning microscopy to visualize colocalization of sporozoites with actin. Actin accumulation occurred in association with internalized sporozoites of both types (Fig. 2).

In addition, disruption of the host actin cytoskeleton with cytochalasin B or cytochalasin D significantly inhibited entry of both genotypes. Pretreatment of HCT-8 cells for 1 h with either microfilament inhibitor substantially reduced infection (Fig. 3A). The reason for the different efficiencies of the two inhibitors is unclear. However, it may reflect differences in the

permeability of the oocyst wall to the drugs since invasion of the cells in the presence of either inhibitor markedly reduced infection regardless of the genotype or inhibitor type (Fig. 3B). Preexposure of oocysts of both genotypes to cytochalasin D and cytochalasin B did not affect subsequent invasiveness.

Pretreatment of oocysts with microtubule inhibitors attenuates cattle but not human genotype invasion of HCT-8 cells.

In order to determine whether *C. parvum* genotype I also requires an intact microtubule cytoskeleton for efficient infection of HCT-8 cells, we examined the effect of the microtubule-depolymerizing agents, colchicine and vincristine, on infection. As described in previous studies (7), inhibition of host cell microtubules did not affect *C. parvum* invasion (data not shown). However, pretreatment of genotype II oocysts with either microtubule inhibitor for 1 h reduced infection of HCT-8 cells in a dose-dependent manner (Fig. 4A and B). Infection in the presence of either 10^{-4} M colchicine or 10^{-4} M vincristine throughout the infection period reduced the in-

FIG. 2. Confocal laser scanning micrographs of host-actin colocalization during invasion of genotype I (A) and genotype II (B) into HCT-8 cells. The green color shows the stained actin, and the red color indicates the intracellular stages of *C. parvum*. Colocalization of host actin and the parasite appears yellow (arrows). Slides were examined by using a laser scanning confocal microscope. Magnification, $\times 400$.

FIG. 3. Effect of cytochalasin D and cytochalasin B on infection of genotype I and genotype II *C. parvum* into HCT-8 cells. Cells were either treated for 1 h with cytochalasin D or cytochalasin B (A) prior to infection or infected in presence of cytochalasin D or cytochalasin B for 48 h (B). All values are given as the mean $\pm$ the standard deviation of triplicate experiments. *, $P < 0.005$ compared to control (no inhibitor).

fection of genotype II oocysts from $88.4\% \pm 1.59\%$ to $30.7\% \pm 1.14\%$ and $7.7\% \pm 2.5\%$, respectively (Fig. 4C). In contrast, neither vincristine nor colchicine treatment of *C. parvum* genotype I oocysts affected the rate of infection of HCT-8 cells (Fig. 4A and B). These data suggest that either the mechanism of host cell entry utilized by *C. parvum* genotype I sporozoites is independent of the parasite's microtubule structures or genotype I oocysts are impermeable to microtubule-depolymerizing drugs used.

Culture of primary human cells and invasion by *C. parvum* genotypes I and II. Isolated cells and crypts attached to the coverslips and spread within 24 to 48 h. The cells grew as isolated epithelial colonies and propagated by growing out from the crypts. The epithelial origin of the cultured cells was demonstrated by immunofluorescence labeling with cytokeratin.

C. parvum genotype II entered primary human cells with an efficiency comparable to the invasion of HCT-8 cells. However, *C. parvum* genotype I infected primary human intestinal cells significantly more efficiently than was the case for HCT-8 cells. In addition, the clustered pattern of infection observed for *C. parvum* genotype I entry into HCT-8 cells was not evident in primary cells, with infection being equally distributed throughout the cell monolayer (Fig. 5).

C. parvum genotype II but not genotype I infects primary bovine intestinal cells.

In order to explore the possibility that the restriction of *C. parvum* genotype I to human hosts reflects species-specific tissue tropism for this genotype, we isolated and infected in vitro bovine intestinal cells. The viability of isolated bovine intestinal cells was monitored by the trypan blue exclusion assay. Microscopically, after 24 to 48 h, at which time they were used for infection studies, these cells appeared healthy and viable. *C. parvum* genotype II infected bovine cells with an efficiency comparable to infection of primary human cells and HCT-8 cells ($92.3\% \pm 1.34\%$). However, genotype I (*C. parvum* 5942) organisms did not infect primary bovine cells. No parasites were seen in association with the bovine cells either adherent (after 1 h without washing) or after a 22-h invasion assay (Fig. 6). In order to corroborate this finding, we used a second *C. parvum* isolate. As observed with isolate 5942, TU502 efficiently invaded primary human cells but did not infect primary bovine intestinal cells (data not shown).

DISCUSSION

Studies of the pathogenesis of *C. parvum* in vitro to date have relied on the use of *C. parvum* genotype II isolates or human-derived isolates of unknown genotype (5–8, 12, 23).

FIG. 4. Effect of vincristine (vin) and colchicine (col) on infection of HCT-8 cells by genotype I and genotype II *C. parvum*. Genotype I and genotype II *C. parvum* oocysts were treated with a range of concentrations of colchicine (A) and vincristine (B) for 15 min at 37°C prior to their use to infect HCT-8 cells. (C) HCT-8 cells were infected by genotype II *C. parvum* in the presence of colchicine and vincristine for 48 h.

FIG. 5. Lectin VVL staining of primary intestinal human epithelial cells infected with *C. parvum* genotype I (A) and genotype II (B). Epithelial cells were infected with 2×10^5 oocysts/ml of genotype I and genotype II. *C. parvum* and stained with lectin VVL. Background staining by the lectin VVL also revealed cell morphology. The efficiency and pattern of infection was similar for both *C. parvum* genotypes ($96.2\% \pm 0.36\%$ for genotype I and $97.4\% \pm 0.1\%$ for genotype II). Slides were examined by using a fluorescence microscope. Magnification, $\times 400$.

Given that *C. parvum* genotype I is the predominant genotype in the majority of studies of sporadic and outbreak cases of human cryptosporidiosis (15, 16, 24, 25), it is essential to characterize and compare its pathogenesis with that of *C. parvum* genotype II.

In the present study a combination of established in vitro tissue culture and an ex vivo primary intestinal cell model of *Cryptosporidium* infection was used to compare infection by *C. parvum* genotype I and genotype II. The data presented here indicate that *C. parvum* genotype I differs from genotype II with regard to the efficiency and pattern of infection and susceptibility to microtubule depolymerizing drugs. In addition, we have shown that *C. parvum* genotype I isolates are unable to infect bovine intestinal cells ex vivo, indicating that host cell tropism underlies species restriction among human and bovine genotypes of *C. parvum*.

Primary intestinal cells have a number of advantages over immortalized tissue culture cell lines for the study of host-parasite interactions in vitro. As a biologically meaningful surrogate model of direct challenge studies in vivo, primary cells have provided important novel insights into other gastrointestinal pathogens, including *Campylobacter* and *Helicobacter* spp. (9, 26). Comparison of *Cryptosporidium* infection of primary and conventional cell lines in the present study demonstrates the potential usefulness of primary cells for the study of *C. parvum* infection. For example, our results indicate that the apparent efficiency of *C. parvum* infection in vitro may be critically dependent on the infection model used. A previous study suggested that genotype I appeared to be more aggressive in its growth in HCT-8 cells than genotype II (19). However, in the present study, entry of a *C. parvum* genotype I isolate into human-derived HCT-8 cells was less efficient than

FIG. 6. Infection of primary bovine intestinal cells with genotype I *C. parvum* isolate 5942 (A) and genotype II *C. parvum* (B). Whereas genotype II *C. parvum* efficiently invaded bovine cells, genotype I did not. Slides were examined by using a fluorescence microscope. Magnification, $\times 400$.

that of a genotype II isolate, and the genotype I isolate showed a distinct, focal pattern of infection in the monolayer. The reason for this difference in pattern of infection of HCT-8 cells by genotype I and genotype II *C. parvum* is uncertain, but it may reflect different adhesin-receptor interactions subserving invasion by the two genotypes of these immortalized cells. On the other hand, in a primary human cell model the two isolates were indistinguishable in terms of the pattern and efficiency of infection. More importantly, our primary cell model clearly shows that bovine cells are resistant to infection by *C. parvum* genotype I.

In order to avoid the confounding effect of variation in isolate pathogenicity (30, 31, 42) on the interpretation of our results, we used two geographically distinct isolates of *C. parvum* genotype I for studies on primary cells. Neither isolate could enter primary intestinal bovine cells. Both entered primary human small bowel cells with similar efficiency. That the observed tropism was not simply a result of host cell adaptation is underlined by the fact that genotype I isolate, TU502, was extensively passaged through gnotobiotic neonatal pigs (a species successfully infected with *C. parvum* genotype I experimentally) before use.

The results of previous studies of the effects of microtubule inhibitors on *Cryptosporidium* infectivity have been contradictory (7, 48). Our data support existing reports indicating that the host microtubule cytoskeleton is not involved in *C. parvum* infection (7). In addition, our results are in agreement with those of Wiest et al. (48), who showed that pretreatment of *C. parvum* oocysts with colchicine and vincristine attenuated infection in a dose-dependent manner. However, in the present study the infectivity in vitro of a *C. parvum* genotype I isolate was not affected by preincubation with colchicine or vincristine. It is possible that genotype I does not require its microtubule cytoskeleton for cell invasion. However, an alternative explanation could be a variation in oocyst permeability of the two *C. parvum* genotypes. Whether isolates of *C. parvum* differ in their requirement for microtubule function during the infection process or vary in their sensitivity to the effects of inhibiting agents requires further study. However, these data suggest that the parasite's microtubule structure is unlikely to be an attractive target for developing novel human anticryptosporidial therapies.

It is possible that *C. parvum* genotype II uses a pathway distinct from that of *C. parvum* genotype I for infection of both human and bovine intestinal cells. Alternatively, *C. parvum* genotype II may have evolved a specialized mechanism of host-parasite interaction specific for infecting bovine intestinal cells that is not shared by *C. parvum* genotype I. Other apicomplexan invasion mechanisms have been shown to have a high degree of redundancy (29, 32). It is possible that genotype I has become specifically adapted for human cells and therefore has less redundancy in invasion pathways than genotype II. Our studies with microfilament inhibition and confocal microscopy showed that, like *C. parvum* genotype II (6, 7), the human genotype requires the participation of the host cell actin cytoskeleton for entry into cells. This, together with the finding that *C. parvum* genotype I did not attach to bovine intestinal cells, strongly suggests that the species tropism we observed occurred at the level of receptor-ligand interaction during the initial stages of infection. Exploration of potential

sporozoite lectin-carbohydrate interactions (7) that subserve these distinct attachment specificities will be an important area for future investigation.

C. parvum genotype I isolates have only rarely been described in nonhuman primates, sheep, and dugongs (14, 27, 39, 46). Under experimental conditions genotype I isolates can infect gnotobiotic piglets, and mixed (genotype I and II) infections have been described following propagation studies in calves (41). However, from an epidemiological perspective this subtype of *C. parvum* is considered to infect humans almost exclusively (45). Under both clinical and experimental conditions *C. parvum* genotype I and II isolates maintain a separate reproductive cycle, indicating a lack of genetic recombination between them (45). Based on these and other molecular genetic and biological data (28), there now exists compelling evidence that *C. parvum* genotype I is a separate species from *C. parvum* genotype II. Our results showing species-restricted host cell tropism adds further support to the proposal that *C. parvum* genotype I should be redesignated as a novel species, *Cryptosporidium hominis* (28, 45).

In conclusion, the data presented here show that the pathogenesis of *C. parvum* genotype I differs from that of genotype II. *C. parvum* genotype I was less infective in HCT-8 cells, and its infectivity was not affected by the addition of microtubule depolymerizing drugs. Furthermore, *C. parvum* genotype I was not able to infect primary bovine intestinal cells, indicating that the host species restriction of this genotype reflects tissue tropism in vivo. It will be important now to investigate and compare with *C. parvum* genotype II the relative effects of *C. parvum* genotype I on signal transduction events (6, 7, 11, 12), cell death (5, 8), and the inflammatory response (20) in existing immortalized cell models and primary intestinal cells.

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