The piglet acute diarrhea model for evaluating efficacy of treatment and control of cryptosporidiosis

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ABSTRACT

Cryptosporidium spp. are ranked as the second leading pathogens causing life-threatening diarrhea in children under 2 years of age. Although *Cryptosporidium hominis* causes three quarters of the cases of cryptosporidiosis, studies on *C. hominis* are limited since natural disease due to *C. hominis* is host-restricted to humans only. In this mini-review, we demonstrate the successful adoption, propagation, and utility of the *C. hominis* strain TU502, isolated originally from an infant with diarrhea in Uganda, in gnotobiotic piglets. The TU502 *C. hominis* strain and the gnotobiotic piglet model currently are the only available preclinical tools to evaluate therapeutics that specifically target *C. hominis*. Infection in this gnotobiotic piglet model displays similar clinical symptoms of diarrhea observed in humans. Here we further describe how this unique model of acute diarrhea, can be used for drug discovery and testing of vaccine candidates against cryptosporidiosis. The shared anatomical, physiological and immunological characteristics between piglets and human infants makes the model ideal for evaluating the efficacy of therapeutics and vaccines against cryptosporidiosis as they become available.

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Introduction

Cryptosporidium spp. are enteric protozoa commonly found in all classes of vertebrates worldwide; they induce diarrheal disease in humans and in newborn ruminants. *Cryptosporidium* spp. are ranked as the second leading pathogens causing life-threatening diarrhea in children under 2 years of age in developing countries, causing an estimated 7.6 million cases of diarrhea and 202,000 deaths annually in sub-Saharan Africa and South Asia.^{1–4} *Cryptosporidium hominis* and *Cryptosporidium parvum* are the species responsible for ~ 75% and ~ 20% cases of illness in humans, respectively. *C. parvum* has a broader range of hosts, and considered zoonotic, whereas *C. hominis* is usually anthroponotic.^{2,5}

While the infection in healthy individuals is usually self-limited, lasting 1 to 2 weeks, it can be life-threatening in young malnourished children and in immunocompromised individuals.^{6–8} *Cryptosporidium* can lead to persistent infection in malnourished children in whom the outcome can lead to severe disease with a higher risk of mortality and/or generating a vicious cycle of malnutrition-*Cryptosporidium* infection.^{2,3,9}

The current therapeutic options for *Cryptosporidium* infection are limited and only partially effective. Nitazoxanide (NTZ) is the only FDA-approved drug for the treatment of *Cryptosporidium* in immunocompetent children and adults.^{10,11} Although NTZ shortens the duration of diarrhea and parasite shedding in immunocompetent adults, its efficacy at best is partial in children and is ineffective in immunocompromised patients.^{12–14} Therefore, more effective parasite-specific drugs are urgently needed to treat *Cryptosporidium* infection.

Animal models of *Cryptosporidium* infection are useful to explore the activity of a candidate drug and may help to predict whether the drug will be efficacious in humans. Although many animal models for *C. parvum* infection are available due to its broader range of hosts, the animal models for *C. hominis* infection are very limited. We have used the gnotobiotic (GB) piglet model to study this parasite because the piglet is susceptible to *C. hominis* and *C. parvum*, the two main species that naturally infect humans. This review focuses on the utility of the GB piglet model of acute diarrhea model for investigating the human *C. hominis* species to evaluate the efficacy of therapeutic candidates.

The gnotobiotic piglet: a model of cryptosporidiosis

Although no animal model ever perfectly mimics the human condition, the pig has emerged as a superior non-primate experimental animal model because of its much closer resemblance to the human infant anatomy, physiology and dietary requirements. Due to such similarities pigs are used extensively as an experimental model for various research investigations including dentistry, ophthalmology, the integumentary, renal systems, the cardiovascular system, and digestive anatomy and physiology.^{15,16} It is also used extensively in organ and xenotransplant studies. This helped generate many of the immune reagents for swine currently available for mice and humans. Pigs are also the only widely utilized animal model that is truly omnivorous, and they have strikingly similar nutritional requirements to those of humans.¹⁶ Additionally, the size of piglets

compared to rodents facilitates handling, observing clinical manifestation of disease including diarrhea, anorexia, dehydration and weight loss, bleeding, surgery, repeated sampling, measuring of respiratory rates, and collection of intestinal content and intestinal epithelial cells for transcriptome analysis.

The GB piglets are derived by Caesarian section and maintained under germ-free conditions, i.e. free of indigenous pathogens and commensals. GB piglets have no interfering maternal antibody, as they are born agammaglobulinemic and fed sterile milk baby formula instead of colostrum. Conventional piglets normally acquire their maternal immunity through intake of colostrum during the first 36 hours after birth. Although full maturation with CD8⁺ T cells and IgA⁺ B cells occurs within 7 weeks, lymphoid structures within GALT develop an adult architecture by 10–15 days, and are populated by CD4⁺ lymphocytes and IgM producing B between 2–4 weeks.¹⁷

Due to lack of maternal antibody and premature gut immune system, GB piglets are very susceptible to human enteric pathogens such as rotavirus,¹⁸ Shiga toxin producing *Escherichia coli*,^{19–21} *Shigella* spp.,²² *Clostridium difficile*,²³ and *Cryptosporidium* spp.^{24–26} The clinical symptoms and the mucosal lesions induced, location and distribution by these agents in GB piglets, are similar or identical to those observed in humans.

Animal models of *C. parvum* include most rodents and newborn calves as well as piglets, that we and others have reviewed elsewhere.^{27–29} However, rodent models are either genetically immunodeficient or require considerable immunosuppressive conditioning to have *C. parvum* infection and seldom show symptoms including diarrhea, which is a major clinical manifestation in human cryptosporidiosis. Calves are naturally infected by *C. parvum* and develop diarrhea. However, calves weigh 50–75 times more than piglets, with large variation of size and availability of one animal at a time may limit the use of experimental groups of animals of the same age, source, size, number, etc. In contrast 10–12 piglets born per litter of siblings with similar size which can be subdivided into 2–3 groups in an experiment.

GB piglets are clinically susceptible to the two-main species that naturally infect humans of all ages, namely *C. hominis* and *C. parvum*.^{30,31} They are also susceptible to the avian species *C. meleagridis* which is often isolated from human patients.³² In GB piglets, *Cryptosporidium* infection induces acute diarrhea with anorexia often dehydration in severe cases and reduced body weight gain. Symptoms last 5–7 days after the onset of diarrhea with corresponding reduced oocyst excretion in feces. The symptoms of *C. hominis* are milder in piglets than those of *C. parvum*.

Maintenance of *C. hominis* TU502

Although *C. hominis* is the species most commonly found in ~ 75% of children in low to moderate income countries where such studies were performed,^{5,33} *C. parvum* is used almost exclusively in the laboratory and field investigations of cryptosporidiosis. *C. parvum* infects all mammals including humans while *C. hominis* naturally infects only humans. Consequently, all in vitro and in vivo studies on cryptosporidiosis are

performed using *C. parvum* as surrogate. Our laboratory successfully adopted the strain TU502 of *C. hominis* obtained from a human patient with diarrhea to propagate in GB piglets.²⁶ This strain has been maintained in our laboratory ever since, through numerous passages (every 8–10 weeks) in piglets. In 2003 and 2016 the genome of TU502 was respectively, partially and then fully sequenced.^{34,35} In 2006 TU502 was evaluated in human volunteer challenge studies,³⁶ displaying clinical characteristics quite distinct from *C. parvum*.

Experiment scheme

C. hominis strain TU502 is propagated in GB piglets for oocyst production which our lab makes available to the scientific community. It is the only source of well characterized reference *C. hominis* strain currently available. GB piglets are sacrificed when the oocyst excretion is highest in feces, and the contents and the mucosal lining of the cecum and the spiral colon are extracted under sterile conditions. The feces are also collected during the days of oocyst excretion. The intestines and feces are kept at 4°C up to 10 weeks and used for oocyst purification and propagation.

GB piglets are derived germfree by Caesarian section and kept inside sterile isolators in individual cages for the duration of the experiments. They are monitored closely over the first 24 hr and only healthy piglets are assigned for an experiment and challenged each with one to five million oocysts of *C. hominis* TU502. Because oocysts purified from intestines appear to be more infectious than the oocysts purified from feces, one million oocysts from intestines or five million oocysts from feces are used as the challenge dose. Figures 1, 2 and 3 show little or no significant clinical difference among one million to five million-oocysts challenged piglets.

To evaluate the efficacy of anti-cryptosporidial drugs, we begin treatment with the onset of diarrhea and/or oocyst excretion in the feces, which occur mostly on day 3 post challenge. The impact of drug treatment is evaluated based on measurable parameters in vivo that include the duration and severity of diarrhea, change of body weight, and the rate of oocyst excretion in feces. After euthanasia a trained board certified veterinary pathologist examines histologically sections of the gastrointestinal tract for *Cryptosporidium* colonization and mucosal lesions. Colonization and mucosal lesions grading are performed blinded and then converted to scores, described in detail below. This eliminates bias and allows statistical comparisons amongst possible experimental groups: infected only; infected and diluent-treated; infected and drug-treated; uninfected and drug-treated; and uninfected untreated.

All animal studies must be approved by the institutional animal care and use committee (IACUC) guidelines.

Clinical observations

Clinical signs of illness are monitored twice daily and symptoms of diarrhea are scored from 0 to 4 (Table 1). Usually, two lab personnel monitor the piglets and score the rate of oocysts excretion in the feces, and the outcome is averaged. Body weight is measured daily.

Figure 1. Fecal excretion of *C. hominis* oocysts after challenge. (A) Daily oocyst excretion and (B) Accumulated counts of oocyst excretion. Piglets were inoculated orally with *C. hominis* TU502 oocysts two days after birth. Rectal swabs were processed for oocyst count. The plot (A) represents mean $\pm$ SEM and the plot (B) represents with box and whiskers (min to max). Two-way ANOVAs with Sidak's multiple comparisons test (A) and Mann-Whitney test (B) were conducted using Graphpad Prism 7.03. No significance between groups was observed. A group of piglets infected with *C. hominis* TU502 1×10^6 oocysts purified from intestinal contents (n = 26, closed circle ●); A group of piglets infected with *C. hominis* TU502 5×10^6 oocysts purified from fecal collection (n = 9, closed square ■).

Figure 2. Diarrhea scores observed in piglets following oocyst challenge. Piglets were inoculated orally with *C. hominis* TU502 oocysts 2 days after birth. The clinical signs of cryptosporidiosis were monitored daily and diarrheal symptom were scored as described in Table 1. The plot represents mean $\pm$ SEM. One-way ANOVA with Tukey's multiple comparisons test were conducted using Graphpad Prism 7.03. **p < 0.01. Although there were significant differences between *C. hominis*-infected groups and a non-infected group, no significant difference was observed between *C. hominis*-infected groups. A group of piglets infected with *C. hominis* TU502 1×10^6 oocysts purified from intestinal contents (n = 26, closed circle ●); A group of piglets infected with *C. hominis* TU502 5×10^6 oocysts purified from fecal collection (n = 9, closed square ■); non-treated group (n = 6, closed triangle ▲).

Untreated control and treated uninfected control groups of piglets are compared with treated infected groups in terms of onset, extent and duration of diarrhea and other symptoms such as anorexia and weight loss, often seen with *C. parvum*, less with *C. hominis* challenge in which symptoms appear to be milder (Figure 2). The onset of diarrhea is usually observed 3 to 4 days post *C. hominis* challenge and the diarrhea normally lasts 7 to 10 days. Moderate diarrhea with yellow or yellow-white and mucoid to watery stool, is usually observed for 3 to 5 days after the onset of diarrhea.

Although all piglets gain body weight, the *C. hominis*-infected piglets invariably have a reduced body weight gain as compared to uninfected animals (Figure 3).

Figure 3. Body weight gain after drug treatment of *C. hominis*-infected piglets. Piglets were inoculated orally with *C. hominis* oocysts two days after birth. Body weight was measured daily. The plot represents mean $\pm$ SEM. One-way ANOVA with Tukey's multiple comparisons test was conducted using Graphpad Prism 7.03. No significance among groups was observed. A group of piglets infected with *C. hominis* TU502 1×10^6 oocysts purified from intestinal contents (n = 26, closed circle ●); A group of piglets infected with *C. hominis* TU502 5×10^6 oocysts purified from fecal collection (n = 8, closed square ■); non-treated group (n = 6, closed triangle ▲).

Fecal sampling for parasite load and drug pharmacokinetics

Rectal swabs are collected daily and processed for analyses of oocyst excretion and for pharmacokinetics (Figure 4). Sampling of rectal swabs is often the only way to collect the fecal material because direct fecal collection is often unsuccessful in diarrheic animals or lack of fecal material at the time of collection. The rectal swabs are placed in 500 μ l sterile H₂O, incubated at room temperature for 10 min; agitated eluates were centrifuged at 13,000 $\times$ g for 10 min to separate the oocysts in the pellet from the residual drugs in supernatant. 300 μ l of supernatant is transferred to a new tube and stored at -80°C for pharmacokinetic study. After the pellet is resuspended with 300 μ l sterile H₂O, 50 μ l and 200 μ l are transferred into new tubes for oocyst counting and DNA extraction, respectively. In human and

Table 1. Scoring system for diarrhea and colonization and mucosal lesion in intestines.

Scores	Description
<i>Diarrhea scores</i>	
0	no diarrhea
1	brown or grey colored, soft stool, mild diarrhea
2	brown to yellow colored, mucoid, small volumetric stool, mild to moderate diarrhea
3	yellow colored, mucoid to watery, medium volumetric stool, moderate diarrhea
4	yellow to white colored, watery, high volumetric stool, severe diarrhea
<i>Colonization scores</i>	
0	no infection detected
1	1–20% epithelial surface infected
2	21–40% surface infected
3	41–60% surface infected
4	61–80% surface infected
5	81–100% surface infected
<i>Mucosal lesion scores</i>	
0	normal
2.5	equivocal
5	mild
10	moderate
15	marked

veterinary clinical situations, oocysts are shed in large numbers in diarrhetic feces. In the gnotobiotic piglet model, we sample with rectal swabs for experimental uniformity and timing, as diarrhetic feces from individual piglets are not always available. While the swab counts of oocysts may be less than ideal, compared with total fecal dry matter, this is adequately compensated by fecal quantitative PCR. Further, we employ additional methods to assess the efficacy of therapy, such as *in vivo* colonization by intracellular parasitic forms, and health of the gut mucosa, when the piglets are euthanized.

Evaluation of *Cryptosporidium* excretion in feces

For oocyst counting, the 50 µl transfer is washed once and 10 µl of 50 µl is mounted on well glass slides (diameter 7 mm); and stained by the modified Kinyoun acid fast method.^{24,37} The number of oocysts are counted in 30 fields under 1,000x magnification. The number of oocysts are counted independently by two lab individuals and averaged. The oocyst counts in feces were high from days 4 to 10

Table 2. Primers and probes used for real time PCR.

Target	Sequence	bp
<i>Heat Shock Protein 70 gene</i>		
Forward	5'-TCT GAA GGA ATG CGA ACA ACT-3'	21
Reverse	5'-GGG TTT GTG ATT GCT TGT CTT T-3'	22
FAM probes	FAM-TGG GCA GAG ATT GGT TGG TGA AGT -IABkFQ	24
<i>18S rRNA gene</i>		
Forward	5'-GCT TTA GAC GGT AGG GTA TTG G-3'	22
Reverse	5'-CTT AGA TGT GGT AGC CGT TTC T-3'	22
FAM probes	FAM/TTA GGG TTC GAT TCC GGA GAG GGA -IABkFQ	24

post challenge and then gradually declined in the TU502 infected and untreated piglets unless an appropriate treatment is commenced (Figure 1).

Cryptosporidium DNA in feces are extracted from the 200 µl transfer with 5 times freeze-thaw cycles and conventional DNA extraction methods and stored at -20°C until quantitative real time PCR. For quantitative real-time PCR, the primers and the probes targeting *Cryptosporidium* Heat Shock Protein 70 gene or 18S rRNA gene (Table 2) are prepared as the premixed primers/probe of primers (5 µM each) and probe (2.5 µM). For PCR reaction, the followings are added to be 10 µl total in a well: 5 µl of PrimeTime® Gene Expression Master Mix (Integrated DNA Technologies, Inc.); 1 µl of the premixed primers/probe; 2 µl of sample DNA; and 2 µl of H₂O. Each sample is prepared in triplicate. PCR was performed with the following condition: polymerase activation 95°C, 20 sec and amplification 95°C, 1 sec and 60°C, 20 sec for 40 cycles. To generate the standard curve, DNA are prepared from TU502 oocysts counted by hemocytometer. The DNA amounts equivalent to *Cryptosporidium* oocysts are calculated from cycle threshold (C_T) of samples using the equation of standard curve. The quantitative PCR analysis shows that *Cryptosporidium* DNA in feces are similar patterns to the oocyst counts in feces.³⁸

Pharmacokinetics

Pharmacokinetic analyses are performed on blood, gut contents, and fecal materials of animals dosed with therapeutics after administration for the duration of the treatment. The measurement of the drug and its metabolites in blood, gut

Figure 4. Process of rectal swabs. Rectal swabs are collected daily and processed for microscopic oocyst counts, quantitation of *Cryptosporidium* DNA, and pharmacokinetics.

contents, and fecal materials help our understanding how these levels correlate with recovery from symptoms of disease following the oral challenge with *C. hominis*. Sera are collected on the designated dates once drug treatment commenced. Fecal eluates are acquired daily from rectal swabs post treatment. Gut contents from cecum and colon are collected on termination.

Although the drug concentrations in fecal eluates are expected to be lower than feces itself, the sampling of rectal swabs are easy to obtain as acquired compared to fecal collection, which is often unsuccessful from animals suffering diarrhea or constipation. We could constantly measure the concentration of azithromycin and the metabolites of NTZ

in the fecal eluates collected daily during the course of treatment,²⁴ providing feasibility and proof of concept that drug in fecal eluates is useful for pharmacokinetic studies.

Hematology and histopathology

Piglets are overdosed with anesthesia, terminally bleed, and euthanized at the designated time points after the onset of drug treatment. Blood samples are analyzed for hematology and serum biochemical data (Table 3). The serum chemistry changes

Table 3. Serum biochemical and hematological data from blood in GB piglets.

Measure	Units	<i>C. hominis</i> only (n = 27)	None (n = 6)	P value
Electrolytes				
Sodium	mEq/L	132.8 ± 1.08	137.0 ± 1.34	0.0914
Potassium	mEq/L	4.0 ± 0.10	4.0 ± 0.15	0.852
Chloride	mEq/L	93.0 ± 0.96	97.3 ± 0.80	0.0466*
Osmolarity	mmol/L	264.8 ± 2.04	272.5 ± 2.84	0.1005
Acid:Base status				
Bicarbonate	mEq/L	22.4 ± 0.57	20.8 ± 1.30	0.2617
Anion Gap	mmol/L	17.4 ± 0.55	18.8 ± 1.66	0.325
Mineral				
Calcium	mg/dL	9.6 ± 0.09	9.6 ± 0.21	0.8825
Phosphorus (Pi)	mg/dL	7.5 ± 0.24	8.3 ± 0.40	0.1547
Magnesium	mg/dL	2.4 ± 0.08	2.2 ± 0.10	0.4225
Renal function				
BUN (urea nitrogen)	mg/dL	1.7 ± 0.11	2.0 ± 0.26	0.2389
Creatinine	mg/dL	0.5 ± 0.02	0.5 ± 0.04	0.5198
Liver function				
Bilirubin	mg/dL	0.4 ± 0.03	0.5 ± 0.04	0.5385
Metabolites				
Cholesterol	mg/dL	96.8 ± 3.40	97.3 ± 3.47	0.9433
Glucose	mg/dL	173.3 ± 8.37	177.0 ± 20.60	0.8541
Triglycerides	mg/dL	56.6 ± 4.24	107.2 ± 17.72	0.0002***
Enzymes				
ALT	U/L	56.1 ± 1.54	49.7 ± 3.33	0.0834
ALP	U/L	1715 ± 124.2	2084 ± 185.6	0.1963
Amylase	U/L	3154 ± 159.6	3758 ± 223.4	0.0958
AST	U/L	62.0 ± 16.12	43.8 ± 3.77	0.6048
Creatine Kinase	U/L	529.7 ± 153.20	399.3 ± 49.29	0.6954
GGT	U/L	57.4 ± 3.10	71.5 ± 7.77	0.0693
Proteins				
Protein (total)	g/dL	2.2 ± 0.08	1.8 ± 0.03	0.0155*
Albumin	g/dL	1.3 ± 0.05	0.9 ± 0.05	0.0056**
Globulin	g/dL	1.0 ± 0.04	0.9 ± 0.05	0.4179
A/G ratio		1.4 ± 0.07	1.1 ± 0.13	0.0768
Hematology analytes				
RBC	M/uL	4.38 ± 0.143	4.66 ± 0.224	0.3532
Hemoglobin	g/dL	7.58 ± 0.237	7.80 ± 0.425	0.6688
Hematocrit	%	25.2 ± 0.71	26.3 ± 1.41	0.4686
MCV	fL	57.82 ± 0.665	56.56 ± 0.832	0.3604
MCH	pg	17.33 ± 0.183	16.70 ± 0.272	0.1106
MCHC	g/dL	30.02 ± 0.239	29.56 ± 0.367	0.3635
RDW	%	19.24 ± 0.293	18.30 ± 0.251	0.1182
WBC	K/uL	7.122 ± 0.373	5.599 ± 0.6203	0.0628
Seg Neuts	%	60.0 ± 2.50	44.3 ± 6.68	0.0148*
Lymphocytes	%	36.6 ± 2.48	47.7 ± 6.75	0.0805
Monocytes	%	1.5 ± 0.34	1.0 ± 0.26	0.4803
Seg Neuts	K/uL	4.956 ± 0.5518	2.922 ± 0.6147	0.1044
Lymphocytes	K/uL	2.643 ± 0.1684	2.498 ± 0.2704	0.7071
Monocytes	K/uL	0.1147 ± 0.0232	0.05333 ± 0.0126	0.2386

The data represent mean ± SEM. Unpaired t-test was conducted between *C. hominis*-infected piglets and non-treated piglets using Graphpad Prism 7.03. ALT: Alanine aminotransferase; ALP: Alkaline Phosphatase; AST: Aspartate aminotransferase; GGT: γ-glutamyl-transferase; RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; WBC: white blood cells; Seg Neuts: segmented neutrophils

Figure 5. Examples of *C. hominis* colonizing the gastrointestinal tract of GB piglets. GB pigs were infected with *C. hominis* TU502 oocysts and euthanized 9 days after infection. The ileum (A), cecum (B), and colon (C) are colonized by *C. hominis* at the expected locations: Epithelial cells of the surface (back arrows) and glands (black ovals). Images were captured by a digital camera, magnified 600 times normal.

Figure 6. Mucosal lesions in GB piglets infected with *C. hominis* TU502 oocysts, and euthanized 9 days later. Normal duodenal villi (A) compared to mild regional villus blunting (B) magnified 100 times. Normal lamina propria of the spiral colon (C) compared to a *C. hominis* TU502-infected GB piglet with moderate infiltration of the lamina propria by lymphocytes, indicated by the asterisk (D) magnified 400 times. Normal colonic glands (E) compared to a *C. hominis* TU502-infected GB piglet with mild neutrophilic infiltrates in a colonic gland lumen ("crypt abscess"), indicated by the black arrow (F) magnified 400 times. Normal colonic surface epithelium (G) compared to a *C. hominis* TU502-infected GB piglet with mild surface epithelial degeneration and necrosis, indicated by the black oval (H) magnified 600 times.

are most consistent with diarrhea, such as electrolyte loss (low Cl^-), water loss and dehydration (mild elevation in serum albumin), and reduced absorption of triglycerides in the small intestine (low triglycerides). The hematology changes are not specific but are consistent with infection/inflammation (mild neutrophilia). At necropsy, we collect standardized sections of the gastrointestinal tract, lung, liver, kidney, spleen, and brain. The tissues are fixed in formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin.

The *C. hominis* colonization score is an estimate of epithelial cells colonized by *C. hominis* organisms in the small and large intestines (Table 1 and Figure 5).^{24,25} Mucosal lesion scores have been developed and validated in blinded testing by a board certified veterinary pathologist.^{24,38} The mucosal lesion score reflects severity of the microscopic lesions from 5 anatomical sites examined by light microscopy: duodenum, jejunum, ileum, cecum, and spiral colon. From all sections, the histologic changes evaluated are: villus atrophy/blunting;

degeneration/apoptosis/necrosis of epithelium, intraepithelial lymphocytes; infiltration of the lamina propria by immune or inflammatory cells; and abscessation of small intestinal crypts or large intestinal glands. Within each region, each lesion type is graded (normal, equivocal, mild, moderate, marked) and then converted to a score as described in Table 1. The final mucosal lesion score for each GB piglet is a sum. Examples of the histologic changes are shown in Figure 6.

Conclusion

The GB piglet diarrhea model of *C. hominis* has been established and many compounds have been evaluated over the last two decades.^{24,25,39} *C. hominis* infection model using GB piglets is effective for evaluating anti-*Cryptosporidium* drugs because piglets, unlike rodents, develop symptoms of diarrhea in which the onset duration and severity, change in body weight gain, and the rate of oocyst excretion in feces can be

measured and quantitated. The impact of treatment on the infection can also be quantitated in terms of extent and distribution of mucosal lesion and parasite colonization.

The outcome of treatment experiments in GB piglets with *C. hominis* and with *C. parvum* helps establish drug efficacy which can form the basis for pre-IND submission to the FDA for evaluation in Phase I human volunteers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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