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Mechanisms of *Tritrichomonas foetus* Pathogenicity in Cats with Insights from Venereal Trichomonosis

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Almost 20 years has passed since trichomonosis was first recognized as a potential cause of diarrhea in domestic cats. Despite progress in confirming disease causation, developing means for diagnosis, and identifying approaches to treatment of the infection, we still know very little about how this parasite causes diarrhea. With increasing recognition of resistance of trichomonosis to treatment with 5-nitroimidazole drugs, new treatment strategies based on an understanding of disease pathogenesis are needed. In this review, lessons learned from the pathogenesis of venereal trichomonosis in people and cattle are applied to clinical observations of trichomonosis in cats in effort to generate insight into areas where further research may be beneficial.

Key words: Diarrhea; Protozoa; *Trichomonas*; *Trichomonas vaginalis*.

Trichomonads belong to the Parabasalia class of flagellated protozoans. Among the various species of trichomonads thus far identified only a handful are regarded as pathogens.[1] The pathogenic mechanisms of trichomonads are poorly understood, and are most studied for those that cause venereal disease, namely *Trichomonas vaginalis* in humans and *Tritrichomonas foetus* in cattle. *Trichomonas vaginalis* affects over 150 million people worldwide and is the most common non-viral sexually transmitted disease.[2] In women, clinical signs of *T. vaginalis* range from an asymptomatic carrier state to severe vaginitis, cervicitis, endometritis, transient or permanent infertility, and premature labor. *Trichomonas vaginalis* also increases the risk of human immunodeficiency virus (HIV) transmission and has been linked to increased risk of cervical and prostate cancer.[3–5] Most men infected with *T. vaginalis* are asymptomatic carriers or are able to rapidly clear the infection. Bovine *T. foetus* is a venereal pathogen of cattle that causes similar pathology to that observed in *T. vaginalis*-infected women. Strict regulations and artificial insemination practices can be used to control the spread of disease in cattle. However, the infection remains prevalent where natural breeding is practiced and can result in considerable economic losses.[6]

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Abbreviations:

CP	cysteine protease
LPG	lipophosphoglycan
PLA2	phospholipase A2
HIV	human immunodeficiency virus
CHO	Chinese hamster ovary
IL	interleukin
TNFα	tumor necrosis factor α
NF-κB	nuclear factor kappa B
TNFβ	tumor necrosis factor β
MIP-3α	macrophage inflammatory protein 3α
IgG	immunoglobulin G

Although less-well understood, trichomonads also are found as inhabitants of the gastrointestinal tract. Examples include *Tritrichomonas suis* of pigs, *Trichomonas muris* of mice, *Tritrichomonas mobiliensis* of squirrel monkeys,[7] *Tetratrichomonas gallinarum* and *Trichomonas gallinae* of birds,[8] and *Pentatrichomonas hominis* of a variety of vertebrate species. More recently, a feline genotype of *Tritrichomonas foetus* was identified as an inhabitant in the intestinal tract of domestic cats.[9–11] Feline *T. foetus* appears to be somewhat host adapted to the intestinal tract of cats, with only one published report of a reproductive tract infection in this species.[12,13] Feline *T. foetus* is less pathogenic than bovine *T. foetus* when used to experimentally infect the uterus of cows.[14] The feline and bovine organisms are genetically distinct[15,16] and there appears to be no association between *T. foetus* infection in cats and reported exposure to cattle.[17] Thus, feline *T. foetus* is believed to represent a different species and a change in name, to *T. blagburni*, has been proposed in effort to distinguish the feline pathogen from bovine *T. foetus*.[18]

Feline *T. foetus* currently is recognized as a primary cause of large bowel diarrhea in domestic cats.[9–11] Infection occurs by direct fecal–oral transmission. Trichomonosis in cats has a worldwide distribution and is prevalent among cats obtained from high density housing environments such as catteries, shelters, or breeding facilities. Clinically affected cats can display a variety of clinical signs including hematochezia, relapsing malodorous diarrhea, fecal incontinence, proctitis and rectal prolapse.[19–21] Only one treatment is documented

to be effective in eradicating *T. foetus* in cats.[22,23] The 5-nitroimidazole drug, ronidazole, is associated with a narrow margin between safety and efficacy.[24] An increasing number of cats are recognized to be harboring *T. foetus* isolates that are resistant to ronidazole.[25] Identification of new antiprotozoal drugs that are capable of killing *T. foetus* has proven difficult.[10,22,26,27] Therefore, a focus placed on understanding the pathogenesis of *T. foetus* infection in cats may provide needed insight into novel approaches that ameliorate the clinical signs of disease, mainly chronic diarrhea.

Pathogenesis of Trichomonosis

Characteristics of Trichomonads

Trichomonads reportedly lack true mitochondria and instead have primitive organelles called hydrogenosomes that enable anaerobic metabolism. This adaptation allows them to live as lumen dwellers in oxygen-poor mucosal environments such as the urogenital and gastrointestinal tracts of their hosts.[1,28] In these locations, trichomonads live as highly motile trophozoites with a 1-stage asexual life cycle. Trichomonads do not form environmentally stable cysts and hence have limited ability to survive outside of their hosts.[1]

Influence of Lumen Microenvironment

Strong, albeit indirect, evidence suggests that dynamic conditions in the host's lumen environment influence both susceptibility and response to trichomonas infections. For *T. vaginalis* infection in women, the vagina presents a complex and temporally fluctuating microenvironment where factors such as the identity of commensal bacteria, lumen pH, redox balance, and mineral content have been identified as important factors in colonization (Fig 1A). In women, healthy vaginal mucosa is colonized by lactobacilli that contribute to maintenance of an acidic lumen pH by the production of lactic acid.[29] *Trichomonas vaginalis* has been reported to phagocytose bacteria including lactobacilli both in vivo and in vitro.[30–35] Phagocytosis of the lactobacilli is presumed to increase vaginal pH and promote colonization and survival of *T. vaginalis* in the vagina.[33] Phagocytosis of erythrocytes by *T. vaginalis* also has been reported and may provide the parasite with vital nutrients and minerals including lipids and iron.[33,36] Major differences in the urogenital microenvironment between men and women have been postulated to explain a general lack of symptomatology in most men with *T. vaginalis* infection. The urogenital tract of men often is oxidative and rich in zinc, which is unfavorable to trichomonad survival. In contrast, the urogenital tract of

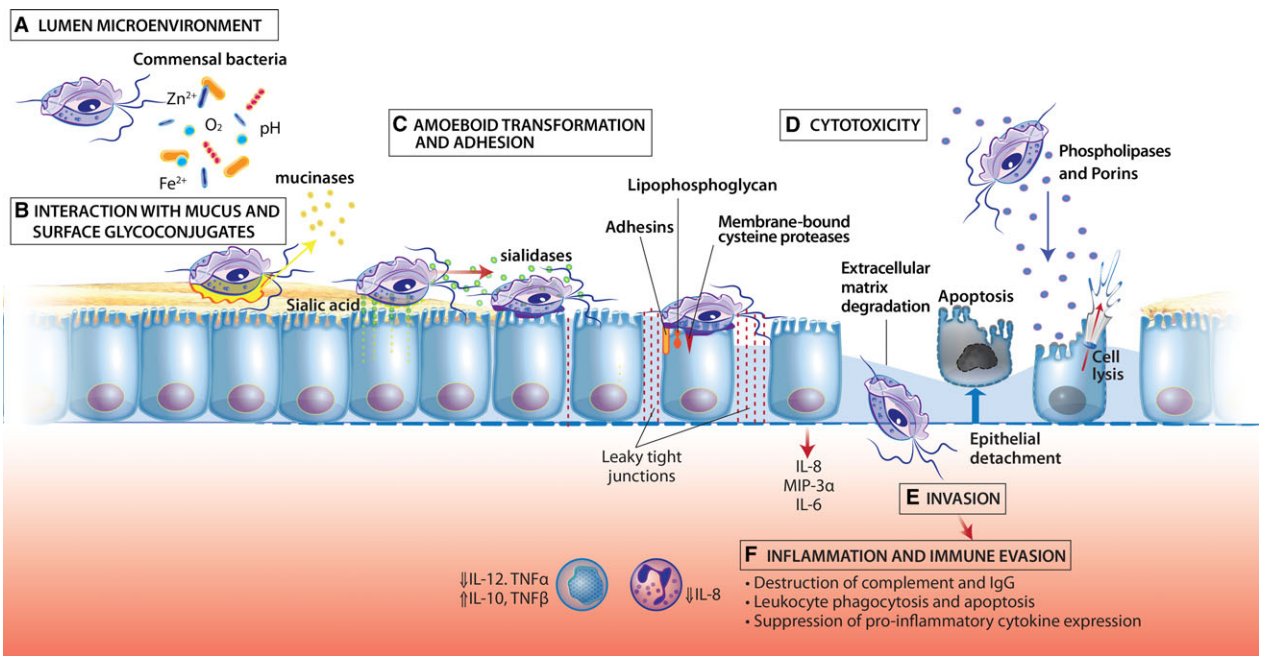

Fig 1. Theorized pathogenic mechanisms of feline *T. foetus* on the basis of clinical and experimental observations of infection and extrapolated from studies of venereal trichomonosis in cattle and people. (**A**) Upon entry into a mucosal environment of the host, trichomonads are influenced by and alter the lumen microenvironment. (**B**) Interaction with surface glycoconjugates and degradation of mucus enables trichomonads to gain access to the surface of the mucosal epithelium. (**C**) Contact with the epithelium results in amoeboid transformation of trichomonads and adhesion to the epithelial cells by means of specific surface-expressed ligands. Contact with the epithelium promotes increases in tight junction permeability, epithelial cell detachment and apoptosis which exposes the lamina propria to invasion by trichomonads (**E**). (**D**) Secretory proteins of trichomonads can directly promote epithelial cell lysis. (**F**) While activating the immune system, trichomonads also exert multiple means to evade the host immune response. Illustration by Alice MacGregor Harvey © North Carolina State University.

women is largely a reducing environment with low zinc and higher concentrations of iron, which favor trichomonad growth and colonization.[37]

Currently, no published studies have investigated the influence of the colonic lumen microenvironment on *T. foetus* infection in cats. Such studies may provide key insights into disease pathogenesis, targets for promoting colonization resistance, or novel approaches to restore normal colonic function. After experimental orogastric infection of kittens, feline *T. foetus* has been demonstrated to colonize the lumen of the ileum, cecum, and colon.[10] In naturally infected cats, massive numbers of trichomonads can be observed in close proximity to the colonic surface epithelium and within the colonic crypts.[38] These trichomonads often are observed in close association with the colonic bacteria (Fig 2).

Circumstantial evidence suggests that colonic bacteria play a key role in the pathogenesis of diarrhea in cats with *T. foetus* infection. For example, diarrhea in cats with trichomonosis often is ameliorated by administration of antibiotics.[9,10,19] Whether these antibiotics diminish a direct bacterial contribution to diarrhea pathogenesis or simply decrease the number of *T. foetus* by depleting their nutrient supply is unclear. Chronically infected cats can experience extended periods of clinical remission, but relapses of diarrhea can be provoked by changes in the diet or stressful events that presumably could alter the colonic microbiota.[19] Anecdotally, the use of "probiotics" has been largely ineffective at ameliorating clinical signs of diarrhea. Based on observations of the predacious relationship between *T. vaginalis* and lactobacilli, it may be relevant to distinguish between bacteria that promote normal colonic homeostasis and those that conceivably could worsen infection by providing a nutrient source for the trichomonads. Compared to bovine *T. foetus*, feline *T. foetus* appears to tolerate a wide range of environmental pH, making this an unlikely target for promotion of colonization resistance.[39] Based on these observations, studies designed to identify specific changes in the colonic microenvironment (including bacterial community composition and lumen mineral content) of cats with trichomonosis and their association with clinical signs of diarrhea are warranted. Such studies may provide a logical rationale for targeted manipulation of the colonic microenvironment to thwart colonization or assuage diarrhea in infected cats.

Colonization of the Intestinal Epithelium

In colonic mucosal biopsy specimens from cats with naturally occurring or experimentally induced *T. foetus* infection, trichomonads frequently are observed in contact with surface mucus or intimately associated with enterocytes along the surface epithelium and crypts[10,38] (Figs 3 and 4). Direct interaction and adhesion of trichomonads to mucus and subsequently the mucosal epithelium is believed to represent a critical first step in establishing colonization and mediating host pathogenicity.[40]

In venereal infections by *T. foetus* in cattle or *T. vaginalis* in women, trichomonads adhere to the vaginal epithelium before invading the uterus and oviducts.[41] The epithelium of the vagina is rich in mucin glycoproteins and antimicrobial factors (such as immunoglobulin and lactoferrin) that serve as barriers against pathogen invasion. In vitro studies indicate that *T. vaginalis* can adhere to and penetrate bovine submaxillary and porcine stomach mucus.[42] In vivo, mucinase activity may allow trichomonads to solubilize the mucus matrix and gain access to the underlying vaginal epithelium (Fig 1B). Ability to evade the immune system and acquire nutrients by proteolytic degradation of immunoglobulins and lactoferrin by trichomonads also has been identified.[43]

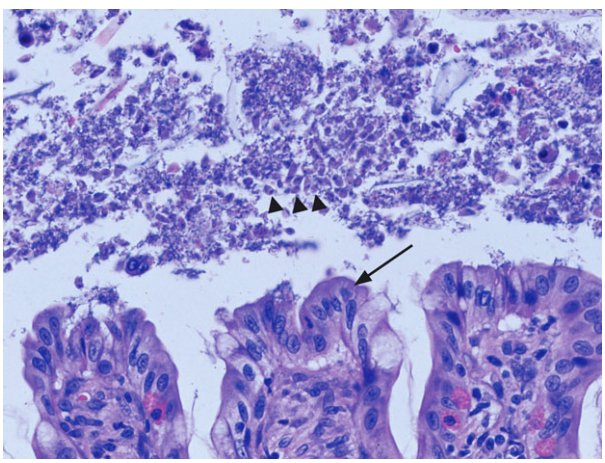

Fig 2. Colonic mucosal necropsy specimen from an 11 month old Persian cat with naturally occurring *T. foetus* infection. The photomicrograph shows the surface epithelium of the colon (closed arrow) with massive numbers of trichomonads (closed arrowheads) admixed with bacteria in the lumen. Hematoxylin and eosin stain. 200× magnification. Figure reproduced from[76] with written permission.

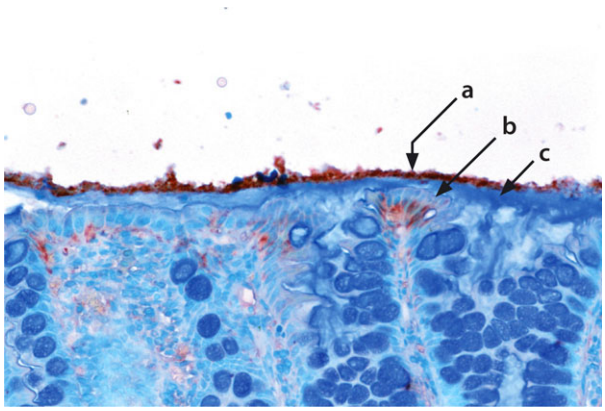

Fig 3. Colonic mucosal biopsy specimen from a kitten experimentally infected with feline *T. foetus*. The specimen was examined by means of immunohistochemistry for detection of trichomonads using a monoclonal antibody (TF1.15) that recognizes a surface antigen of *T. foetus* (a gift from Dr. Lynette Corbiel). Trichomonads (a) are revealed by the red chromogen, 3-amino-9-ethylcarbazole and are observed adhering to a layer of mucus (c) that covers the surface of the colonic epithelium (b). Counterstain is alcian blue. 200× magnification.

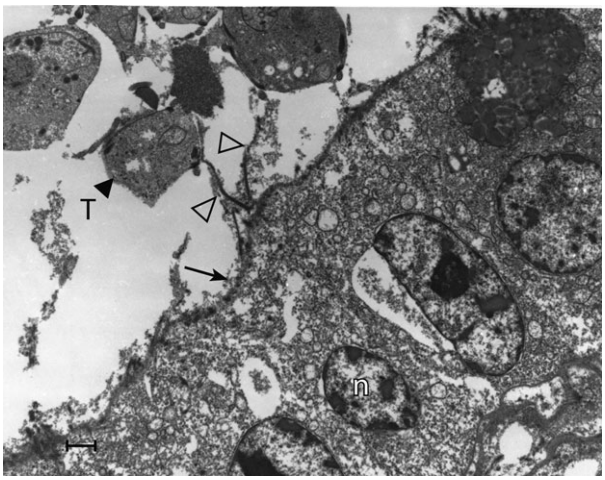

Fig 4. Transmission electron micrograph of a colonic mucosal biopsy specimen from a cat naturally infected with feline *T. foetus*. The figure demonstrates direct interaction between trichomonad flagella (open arrowheads) and the apical surface of colonic epithelial cells (closed arrow). T = trichomonad, n = representative nucleus of an intestinal epithelial cell. Bar = 1 μm.

Upon initial binding to vaginal epithelial cells, signal transduction events upregulate the transcription of *T. vaginalis* virulence genes and stimulate trichomonads to undergo a rapid morphological transformation from a pear-like shape to a flattened amoeboid organism[44,45] (Fig 1C). During this transformation, trichomonads extend short filopodia toward epithelial cells and numerous membrane–membrane contact points can be observed.[45] Amoeboid transformation likely maximizes host cell contact, which is essential for effective adherence in the challenging, secretory environment of the vagina. Adhesion and signaling by trichomonads are host cell- and species-specific. Amoeboid transformation does not take place when trichomonads are in contact with fibroblasts or other "off-target" cell types, and *T. vaginalis* organisms adhere with weaker affinity to bovine vaginal epithelial cells compared to bovine *T. foetus*.[45,46]

Few studies have examined the mechanistic interaction of feline *T. foetus* with intestinal epithelium. A major limitation is a current lack of feline intestinal epithelial cell lines with which to conduct such studies. Recent studies examining *T. foetus* infection in a coculture model with monolayers of porcine intestinal epithelial cells indicate that feline *T. foetus* adheres to the intestinal epithelium and does so with saturable and competitive-binding kinetics[47] (Fig 5). These findings suggest that adhesion of *T. foetus* to the intestinal epithelium occurs by means of specific receptor–ligand interactions. Such interactions theoretically could be outcompeted as a mechanism to abrogate intestinal colonization. Trichomonads possess a number of cell surface molecules that are capable of mediating adhesion to epithelial cells. These include sialic acid-binding lectins, adhesin proteins, lipophosphoglycan, and cellular proteases[46,48–50] (Fig 1C).

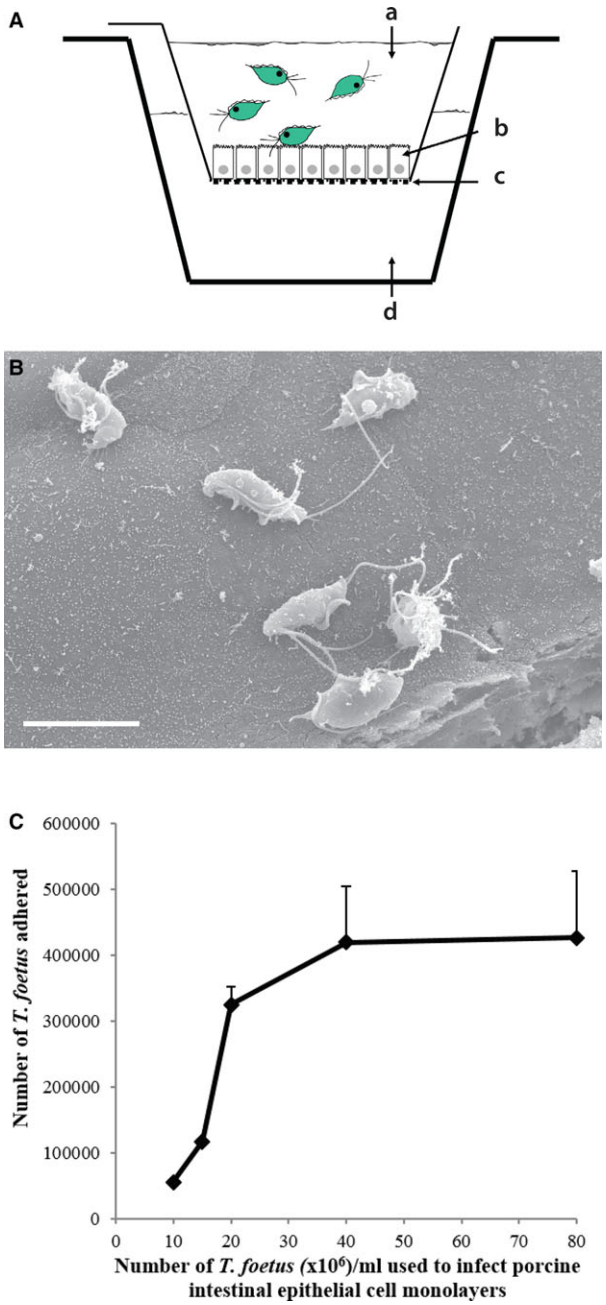

Fig 5. Coculture of feline *T. foetus* with monolayers of porcine intestinal epithelial cells demonstrates saturable binding of trichomonads to intestinal epithelium. Current research approaches as shown in panel **A** use monolayers of intestinal epithelial cells (b) suspended in apical (a) and basolateral media (d) that are separated by means of a semipermeable basement membrane support (c). Panel **B** shows a scanning electron microscopy image of six feline *T. foetus* trophozoites adhering to the surface of a monolayer of porcine intestinal epithelial cells (bar = 10 μm). In panel **C**, ^{3}H-thymidine-labeled trichomonads demonstrate saturable binding when added in increasing numbers to the apical media of monolayers of porcine intestinal epithelial cells. Figure 5B and C reproduced from[47] with written permission.

Sialic Acid-Binding Lectins. The epithelial lining of the reproductive and gastrointestinal tracts are particularly rich in cell surface-expressed glycoconjugates.[51]

Sialic acid, which is a generic name that describes a large family of 9-carbon ketosugars, commonly is expressed as the terminal monosaccharide on these glycoconjugates. Because of their position, sialic acid residues perform a number of important host cell functions including cellular recognition, immune cell communication, and maintenance of mucosal barrier integrity.[52] Thus, it is not surprising that many mucosal pathogens including bovine *T. foetus* have developed mechanisms to disrupt or exploit host sialic acids.[51,53] In vitro, bovine *T. foetus* binds to Chinese hamster ovary (CHO) epithelial cells and bovine cervical mucus using sialic acid-binding lectins. Adhesion of bovine *T. foetus* can be inhibited by desialyation of CHO epithelial cells and mucus.[53] These findings suggest a role for sialic acid in bovine *T. foetus* adhesion. Interestingly, bovine *T. foetus* also expresses its own sialidase activity in both the plasma membrane of the cell body and flagella.[54] A possible explanation for the presence of both a sialic acid-binding lectin as well as sialidase activity is that bovine *T. foetus* uses sialic acid as an initial binding site but later cleaves sialic acid enabling trichomonads to contact epithelial cells using other known adhesins. Similar to bovine *T. foetus*, our unpublished observations suggest that adhesion of feline *T. foetus* to porcine intestinal epithelial cells also is partially dependent on a sialic acid-based mechanism. Adhesion of feline *T. foetus* can be inhibited 50–65% by either removing sialic acid residues from the epithelial cells using exogenous sialidase or by pretreatment of *T. foetus* with exogenous sialic acid or sialidase inhibitors. These pharmacological agents have no direct detrimental effects on survival of *T. foetus* in vitro. We have treated two naturally infected cats with a commercially available neuraminidase (ie, sialidase) inhibitor[a] (4.4 mg/kg PO q12 h for 10 days) and determined the treatment to be ineffective in eradicating the infection (unpublished data). Although such studies are preliminary, they dampen our enthusiasm for targeting sialic acid-based mechanisms for treatment of *T. foetus* infection in cats.

Adhesin Proteins. Under iron-rich conditions and upon contact with epithelial cells, trichomonads express and mobilize adhesin proteins to their cell surface.[45,55] At least one bovine *T. foetus* and five functionally diverse *T. vaginalis* adhesin proteins, named on the basis of their molecular weights (*T. foetus*: Tf190; *T. vaginalis*: AP120, AP65, AP51, AP33, AP23) have been identified.[48,49,56,57] A direct relationship exists between cellular adherence and the abundance of adhesin proteins that remain bound to vaginal epithelial and HeLa cell surfaces after interaction with trichomonads.[49,58,59] Isolates devoid of adhesins or treated with antiadhesin antibodies become nonadherent.[58] When mobilization for adherence is not required, adhesin proteins function as "moonlighting" proteins and can be utilized within the cytoplasm and hydrogenosome for nonenzymatic and enzymatic cellular processes.[58,60]

Comparative studies currently are being undertaken to identify the presence and functionality of adhesin proteins expressed by feline *T. foetus*. Unpublished data suggest that feline *T. foetus* shares at least some protein adhesins in common with bovine *T. foetus* and that immunoblockade of these adhesins abrogates the cytotoxicity of feline *T. foetus* when cocultured with porcine intestinal epithelial cells.[b] These observations suggest a potential role for these adhesins to serve as treatment, vaccination, or diagnostic targets for *T. foetus* infection in cats.

Lipophosphoglycan. In addition to protein adhesins, the surface of trichomonads consists of a dense glycocalyx of carbohydrates and glycolipids.[46,61] Removal of these surface carbohydrates by periodate oxidation abrogates adhesion, which suggests that trichomonad surface carbohydrates are multifunctional, providing both protection to the parasite as well as serving a role in colonization.[46] Lipophosphoglycan (LPG) is the most abundantly expressed glycosylated molecule found on the surface of bovine *T. foetus* and *T. vaginalis*.[61–63] Lipophosphoglycan is anchored to the cell membrane by inositol-phosphoceramide moieties and contains mostly carbohydrate and lipid.[63,64] Several studies implicate LPG in trichomonad adhesion and contact-dependent cytotoxicity to epithelial cells.[65] Purified exogenous LPG significantly inhibits adhesion of bovine *T. foetus* and *T. vaginalis* to host vaginal epithelial cells.[46,50] As of now, studies to determine the presence or function of LPG in feline *T. foetus* have not been reported.

Cysteine Proteases as Adhesins. Like adhesins, cysteine proteases are multifunctional proteins that participate in adhesion of trichomonads to epithelial cells in addition to performing a number of other cellular functions that contribute to the parasite's pathogenicity. Cysteine proteases mediate efficient adhesion of *T. vaginalis* to HeLa cells and vaginal epithelial cells in vitro.[66] In bovine *T. foetus*, the role of cysteine proteases as adhesion proteins has not been investigated directly but is suggested by studies in a mouse model of venereal trichomonosis in which inhibition of trichomonad cysteine protease activity resulted in decreased genital colonization.[58] At least 23 and 15 different cysteine proteases have been identified in *T. vaginalis* and bovine *T. foetus*, respectively.[60,67–70] At least three *T. vaginalis* surface-expressed cysteine proteases (CP30, CP65 and TvLEGU-1) play a key role in adhesion and adhesion-dependent cytotoxicity to cervical and vaginal epithelial cells.[48,66,71–74]

Cysteine proteases possessed by feline *T. foetus* and their role in intestinal colonization are now beginning to be characterized. Eight protein-coding genes for cysteine proteases have been identified in feline *T. foetus* isolates[16] with CP7 being recognized as preferentially transcribed.[75] In vitro studies indicate that feline *T. foetus* has cell-associated cysteine protease activity that promotes adhesion of the parasite to porcine intestinal epithelial cell monolayers. Moreover, adhesion of feline *T. foetus* to the intestinal epithelium can be significantly inhibited using a Clan CA papain-like cysteine protease inhibitor (E-64).[76]

Cytotoxic Effects on Mucosal Epithelium

Trichomonads live in environments lined by a mucous membrane. These mucous membranes are defended by

epithelial cells. Most of the cytopathic effects of trichomonads appear to be initiated by adherence of the parasite to the epithelium. For example, adhesion of *T. vaginalis* to intestinal epithelial monolayers (Caco-2) results in an increase in permeability that is associated with altered tight junction function.[55] When *T. vaginalis* is used to infect vaginal epithelial cells or HeLa cell monolayers, the epithelial cells undergo detachment, lysis, and apoptosis. Inhibition of the adhesion significantly decreases these cytotoxic effects.[77–79] Antiadhesin antibodies inhibit the binding of *T. vaginalis* to host epithelial cells and protect host cells from cytotoxicity.[80] Furthermore, *T. vaginalis* with mutated, truncated LPG adhere 7-fold less and induce significantly less cytotoxicity to ectocervical cells compared to parent strains.[65] Finally, anti-cysteine protease antibodies significantly inhibit adhesion and decrease the cytotoxicity of *T. vaginalis* to HeLa cell monolayers.[71,72]

In the large intestine, the epithelium consists of a single layer of cells and associated tight junctions that create a selective barrier that is responsible for efficient water absorption while simultaneously preventing translocation of colonic bacteria and antigens into underlying host tissues. Several observations suggest that *T. foetus* infection in cats results in injury to the colonic epithelium, as represented by the common clinical sign of hematochezia in affected cats.[9,19,21] Histologically, colonic mucosal lesions in infected cats are characterized by attenuation of the surface epithelial cells and increased crypt epithelial cell mitotic activity. These lesions, which were found to be more severe when associated with the presence of trichomonads, indicate ongoing loss of colonic epithelial cells. In severe cases, epithelial detachment and apoptosis result in loss of epithelial continuity and invasion of trichomonads into the subepithelium[38] (Fig 6). Interestingly, similar subepithelial invasion of trichomonads has been described in the intestinal tract of fetuses aborted from cows infected with bovine *T. foetus*.[81]

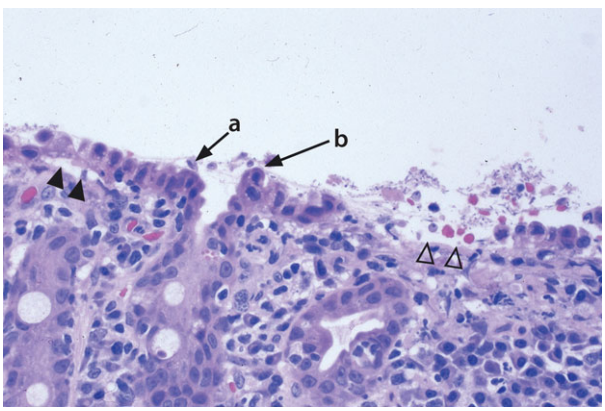

Fig 6. Colonic mucosal biopsy specimen from a cat naturally infected with feline *T. foetus*. There is detachment (closed arrowheads) and apoptotic shedding (b) of the surface epithelial cells, resulting in denuded regions that expose the underlying lamina propria (open arrowheads). The lamina propria is infiltrated with lymphocytes, plasma cells and neutrophils. A trichomonad can be distinguished along the surface epithelium (a). Hematoxylin and eosin stain. 400× magnification.

Cysteine Proteases as Cytotoxic Factors. In addition to mediating epithelial cell adhesion, both surface-expressed and secreted cysteine proteases play numerous indispensable roles in the pathogenicity of trichomonads. Expression of cysteine proteases by trichomonads can be dependent on many environmental factors including availability of polyamines, zinc, and iron.[82,83] Many of the *T. vaginalis* cysteine proteases have been identified in vaginal secretions from infected women.[84,85] The cytotoxic effects of cysteine proteases include degradation of mucus, complement and immunoglobulin, and destruction of extracellular matrix (fibronectin, several types of collagen, and laminin).[43,72,73,84,86,87] Cysteine proteases therefore can function as epithelial cell detaching factors and can mediate direct cytotoxic effects on vaginal and uterine epithelial cells by induction of apoptosis[72,73,84,88–90] (Fig 1D).

Little is known regarding the virulence factors responsible for cytopathogenicity of feline *T. foetus*. However, recent in vitro studies have shown that cysteine proteases mediate adhesion-dependent cytotoxic effects of feline *T. foetus* on porcine intestinal epithelial cells. This cytotoxic effect is associated with activation of apoptosis and can be ameliorated by pretreatment of feline *T. foetus* isolates with a cysteine protease inhibitor (Fig 7).[76] In contrast to feline *T. foetus*, a presumably nonpathogenic feline isolate of *Pentatrichomonas hominis* lacked detectable cysteine protease activity and had no demonstrable cytopathic capabilities. These findings are remarkably similar to the pathogenic effects of feline *T. foetus* on the colonic epithelium in vivo. Ongoing studies are focused on identification of small-molecule cysteine protease inhibitors that could be administered PO to *T. foetus*-infected cats to potentially ameliorate clinical signs of diarrhea.[c] Given the multifactorial and cross-species importance of the cysteine proteases in mediating pathogenic effects of trichomonads, this area appears to be a promising focus for additional study.

Phospholipases and Porins. *Trichomonas vaginalis* produces phospholipases, which like many of the secreted virulence factors, appear to participate in both contact-dependent and independent cytolytic activities[91,92] (Fig 1D). Phospholipase A2 (PLA2) activity has been identified in the soluble fraction of *T. vaginalis* protein extracts. Furthermore, increased PLA2 concentrations have been reported in vaginal secretions of pregnant women infected with *T. vaginalis* where they are speculated to contribute to the pathogenesis of preterm birth.[93] Hydrolysis of phospholipids to free fatty acids by trichomonads supports parasite survival by providing a source of lipid nutrients while also mediating lysis of host cell membranes.[91] *Trichomonas vaginalis* also secretes porins that exhibit a perforin-like activity and induce cellular cytotoxicity by creation of pores in erythrocyte membranes *in vitro*.[94,95] Bovine *T. foetus* is thought to possess endogenous phospholipase C activ-

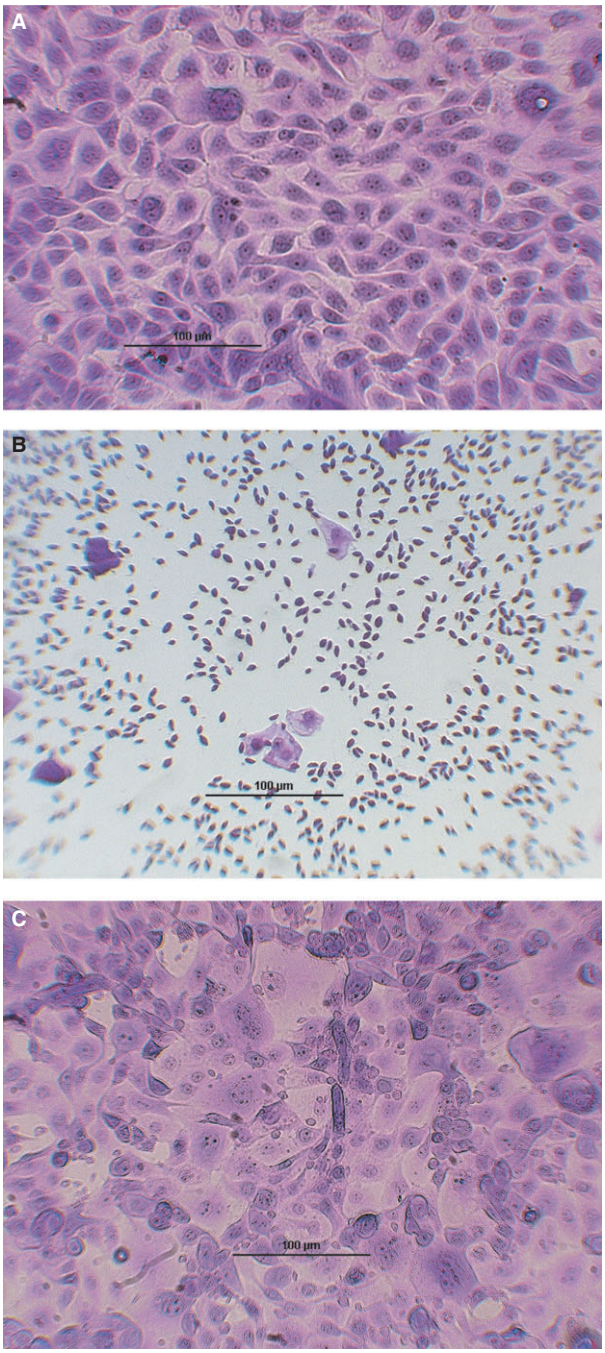

Fig 7. Representative light microscopy image of a confluent monolayer of porcine intestinal epithelial cells before infection with feline *T. foetus* (**A**) and after 24 h of coculture with feline *T. foetus* alone (**B**) or after pretreatment of *T. foetus* with a cysteine protease inhibitor (**C**) 100 µM cystatin from chicken egg white. Only a few epithelial cells remain after infection with *T. foetus* and the image demonstrates numerous trichomonads adhering to the denuded culture dish (B). Pretreatment of *T. foetus* with the cysteine protease inhibitor significantly ameliorates cytotoxicity toward the intestinal epithelium with significant survival of the monolayer after 24 h of infection (C). Crystal violet stain. 200× magnification. Bar = 100 µm.

ity.[54] Investigation into the role of phospholipases and porins in both bovine and feline *T. foetus* infection requires further study.

Host Immune System Activation and Evasion

Trichomonosis generally is characterized by chronic infections. Although both *T. vaginalis* and bovine *T. foetus* can be spontaneously eliminated by their respective hosts, neither infection confers lifelong, acquired immunity. The innate immune system plays a critical role in resisting trichomonad infection, and trichomonads have developed a number of mechanisms to combat and evade the host immune response. These include induction of apoptosis of innate immune cells, modulation of host cytokine expression, degradation of immune cell components, and expression of phenotypic variation (Fig 1F). *Trichomonas vaginalis* has been shown to secrete exosomes that fuse with ectocervical cells in vitro and decrease their secretion of the proinflammatory cytokine interleukin-8 (IL-8).[96] *Trichomonas vaginalis* and bovine *T. foetus* infection are associated with endometrial inflammation characterized by the presence of neutrophils, plasma cells, and lymphocytes.[97,98] In in vitro, *T. vaginalis* has been shown to phagocytose leukocytes and induce apoptosis of neutrophils and macrophages.[33,99,100] Moreover, *T. vaginalis* suppresses expression of the macrophage proinflammatory cytokines interleukin-12 and tumor necrosis factor α (TNFα) by inhibition of nuclear factor kappa B (NF-κB) activity, and enhances expression of anti-inflammatory cytokines interleukin-10 and tumor necrosis factor β (TNFβ).[101,102] Additional resistance of bovine *T. foetus* and *T. vaginalis* to host immune defense is provided by evasion of nonspecific and specific immune cell components by cysteine protease-mediated degradation of lactoferrin, fibronectin, fibrinogen, albumin, immunoglobulins (IgG1, IgG2), and surface-bound complement C3.[43,86,103] In addition, *T. vaginalis* cysteine proteases contribute to trichomonad phenotypic variation and antigenic heterogeneity, providing another layer of protection from host antibodies by degrading prominent surface immunogens and making the once "visible" trichomonad unrecognizable to the host.[104] Despite multiple mechanisms for evasion of the innate immune response, infiltration of the subepithelium with inflammatory cells is a characteristic feature of most trichomonad infections. After colonization, *T. vaginalis* evokes an influx of neutrophils across the vaginal epithelial barrier.[97,98] In particular, LPG isolated from *T. vaginalis* but not bovine *T. foetus* has the ability to stimulate release of proinflammatory cytokines IL-8, macrophage inflammatory protein 3α (MIP-3α), and interleukin-6 from vaginal epithelial cells.[61,64]

In naturally infected cats, similar to human *T. vaginalis* and bovine *T. foetus* infection, feline *T. foetus* infection histologically is characterized by an influx of lymphocytes, plasma cells and neutrophils into the subepithelial lamina propria (Fig 6).[38] The proinflammatory molecules responsible for eliciting intestinal inflammation in *T. foetus* infection are unknown. Immunohistochemical examination of colonic mucosa from naturally infected cats not only shows *T. foetus* organisms in contact with the surface epithelium but

also the presence of immunoreactive *T. foetus* antigen within the epithelial cells and lamina propria.[10,38] Therefore, release of proinflammatory cytokines by the infected intestinal epithelial cells is suspected, but not yet conclusively identified. Moreover, breaches in continuity of the intestinal epithelium and invasion of *T. foetus* into the lamina propria, although uncommon, likely serves as a direct source of immune stimulation. Colonic mucosal inflammation likely contributes to diarrhea in cats infected with *T. foetus*, and this conclusion is supported by complete resolution of colonic inflammation in cats during periods of spontaneous clinical remission.[19] Further evidence, however, suggests that inflammation may not be the only cause of diarrhea in infected cats. After experimental infection of cats with *T. foetus*, diarrhea has been observed to precede development of microscopic inflammation,[10] and diarrhea in naturally infected cats typically fails to respond to treatment with anti-inflammatory drugs (eg, corticosteroids).[19] Therefore, studies to characterize nonimmune-mediated mechanisms of diarrhea in cats with *T. foetus* infection appear warranted, including changes in intestinal epithelial cell barrier and ion transport functions or even influences on colonic motility. Such studies may provide further insights into the cause of diarrhea and therefore strategies to ameliorate the clinical signs of *T. foetus* in infected cats.

Conclusion

Despite its widespread prevalence and lack of consistently effective therapies, feline *T. foetus* has received considerably less attention than venereal trichomonosis. Based on insights from studies of venereal trichomonosis, several areas of focus can be identified that are likely to lead to a better understanding of how feline *T. foetus* causes clinical disease in cats. In our opinion, studies to examine the influence of the microbiome on disease pathogenesis, characterize membrane bound and soluble factors of feline *T. foetus* that are responsible for cytotoxicity, and establish the impact of feline *T. foetus* on the intestinal epithelial cell barrier and electrolyte transport function are likely to result in additional novel therapies to ameliorate clinical signs of disease in affected cats.

Footnotes

[a] Oseltamivir phosphate (Tamiflu), Genentech USA Inc., South San Francisco, CA

[b] Gould E, Brand M, Tolbert MK. Quantitative and qualitative identification of feline *Tritrichomonas foetus* surface antigens: putative targets for diagnosis and treatment (abstract). J Vet Intern Med 2015;29:1179

[c] Tolbert MK, Gould E, Brand M. Feline *T. foetus* cytotoxicity can be inhibited by selective, small-molecule cysteine protease inhibitors (abstract). J Vet Intern Med 2015;29:1273

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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