2015 Miller Trust Grant Proposal for the Winn Feline Foundation

1. Title of Study:

Study Name: Targeting Feline Trichomonosis: Identification and Evaluation of the Role of CP30 in Feline *Tritrichomonas foetus* Cytopathogenicity

2. List ALL Principal Investigator(s) Information:

a. Name: M. Katherine Tolbert, DVM, PhD, DACVIM

Institution: The University of Tennessee College of Veterinary Medicine

Mailing Address:

3. Agency/Institution Information (where grant would be payable):

Agency Name: The University of Tennessee

Mailing Address:

EIN Number (US Applicants):

Check Made Payable to:

Grant Administrator Name:

Grant Administrator Email:

4. Amount Requested:

$ 21775.31

Signatures:

Signature of the principal investigator and appropriate grant administrator:

Signature:

Signature:

Typing your name above constitutes electronic signature.

Scientific Summary Abstract

Feline *Tritrichomonas foetus* (Tf) is a significant cause of infectious colitis in cats worldwide. Despite a high prevalence, a worldwide distribution of infection, and lack of consistently effective drugs for treatment, the cellular mechanisms of feline Tf remain poorly understood. In previous studies, we established an *in vitro* co-culture model system to evaluate the pathogenic mechanisms of feline Tf. Using this model system, we demonstrated that feline Tf produces cysteine proteases (CPs) that are critical to their cytopathogenicity. Broad-spectrum inhibition of Tf CPs significantly ameliorates intestinal epithelial cytotoxicity. However, cats also produce CPs that are part of life-critical systems. Thus, broad inhibition of cysteine proteases would likely be toxic to cats. Previous investigators have shown the importance of a specific CP, CP30, in mediating bovine and human trichomonad cytopathogenicity. Thus, we believe investigation into the presence and role of CP30 in feline Tf represents a promising new direction for treatment of feline trichomonosis. The specific aims of this study are to characterize the presence of CP30 in 6 different feline Tf isolates and to evaluate the effect of targeted inhibition of CP30 on feline Tf CP activity and feline Tf-induced intestinal epithelial cytopathogenicity. We hypothesize that CP30 is expressed by all feline Tf isolates and that targeted inhibition of CP30 will be an effective treatment to ameliorate Tf-induced cytopathogenicity towards the intestinal epithelium.

Lay-language abstract

Feline *Tritrichomonas foetus* (Tf) is a significant cause of infectious diarrhea in cats worldwide. Despite a high prevalence, a worldwide distribution of infection, and lack of consistently effective drugs for treatment, the ways in which feline Tf causes disease are poorly understood. In previous studies, we established a benchtop model to evaluate the ways in which feline Tf causes disease. These studies have allowed us to understand more about Tf infection without necessitating the use of live animals. Using this model system, we demonstrated that feline Tf produces cysteine proteases (CPs) that are critical to their ability to cause disease. Complete inhibition of Tf CPs significantly ameliorates damage to the inner layer of the intestine. However, cats also produce CPs that are important to their health. Thus, complete inhibition of CP would likely be toxic to cats. Previous investigators have shown the importance of a specific CP, CP30, in mediating bovine and human trichomonad infection. Thus, we believe investigation into the presence and role of CP30 in feline Tf represents a promising new direction for treatment of feline trichomonosis. The specific aims of this study are to characterize the presence of CP30 in Tf obtained from 6 naturally infected cats and to evaluate the effect of inhibition of CP30 on feline Tf CP activity and feline Tf-infection using our benchtop model. We hypothesize that CP30 is expressed by all feline Tf and that targeted inhibition of CP30 will be an effective treatment for feline trichomonosis.

Not a continuation study

In preliminary studies, we have demonstrated that feline *Tritrichomonas foetus* (Tf) produce cysteine proteases (CPs) and that targeted inhibition of CP activity ameliorates Tf-induced cytopathogenicity towards the porcine intestinal epithelium. **CP 30 (CP30)** has been identified to be a major virulence factor in bovine and human trichomonad infection and represents a novel target for the treatment of feline trichomonosis.

We **hypothesize** that CP30 is expressed by all feline Tf isolates and that targeted inhibition of CP30 will be an effective treatment to ameliorate Tf-induced cytopathogenicity towards the intestinal epithelium.

Objective 1: Characterize the presence of CP30 in 6 feline Tf isolates cultivated from naturally infected cats.
Objective 2: Investigate the effect of inhibition of CP30 on Tf CP activity and feline Tf-induced intestinal cytopathogenicity.

BACKGROUND AND SIGNIFICANCE: Feline *Tritrichomonas foetus* (Tf) is recognized as a significant cause of diarrhea in domestic cats with a prevalence rate as high as 30%. Feline Tf is a global pathogen. The clinical outcome of feline Tf infection ranges from asymptomatic to a chronic, recurrent, foul-smelling mucoid to hemorrhagic diarrhea.[2] Even with resolution of symptoms, many cats remain persistently infected which likely results in continued spread of the pathogen. Ronidazole is the only treatment identified to be effective in some cats with trichomonosis. Unfortunately, side effects such as neurotoxicity and the development of resistant strains have made treatment of Tf infection with ronidazole increasingly ineffective.[3, 4] Identification of alterative drugs capable of preventing or ameliorating signs of feline Tf infection is dependent on understanding the pathophysiology of feline trichomonosis. However, despite a high prevalence, a worldwide distribution of infection, and lack of consistently effective drugs for treatment, the cellular mechanisms of feline Tf remain poorly understood. Such studies are needed to better understand the pathogenicity of feline Tf and to enable the identification of novel strategies designed to prevent or treat the clinical signs of feline trichomonosis.

Our lab has been dedicated to uncovering the mechanisms of feline Tf cytopathogenicity in order to identify molecular factors that could be targeted for prevention and/or treatment of feline trichomonosis. Because cellular proteases are implicated in the pathogenicity of trichomonads that cause venereal disease (e.g. human *T. vaginalis* and bovine *T. foetus*) as well as enteric protozoal pathogens (e.g. *Entamoeba, Toxoplasma*),[5-8] we focused our attention on investigating the role of proteases in feline Tf infection. In previously published studies, we determined that feline Tf produce both cysteine (CP) and serine proteases (**Fig. 1**).[1] **Using a validated *in vitro* co-culture model of Tf infection, we demonstrated that the pathological effects of feline Tf were chiefly dependent on Tf CP activity.** Tf CP activity enabled cytopathic effects by facilitating adhesion of Tf to the intestinal epithelium. Pharmacologic inhibition of Tf CP activity with a broad-spectrum CP inhibitor significantly inhibited adhesion of Tf to intestinal epithelial cells and largely prevented activation of apoptosis signaling and epithelial monolayer destruction (**Fig. 2**).[1] **These findings suggest that targeting feline CP activity represents a novel approach for the treatment of feline trichomonosis.** However, cats, like all mammals, also produce CPs that are part of life-critical systems (e.g. extracellular matrix turnover, prevention of abnormal cell growth). **Thus, broad inhibition of CPs would likely be toxic to cats.** In order to reduce the risk of host toxicity, anti-CP therapies must be tailored to selectively inhibit the pathogen proteases that are responsible for its cytopathic effects or those that have redundant mechanisms of production or function with the feline host. Investigations into the role of CPs in the cytopathogenicity of the venereal trichomonads, bovine *T. foetus* and human *T. vaginalis*, have revealed that both trichomonads produce a CP (named "**CP30**") that migrates to the 30 kDa region. *In vitro* studies into the role of CP30 demonstrated that it is the major virulence factor responsible for

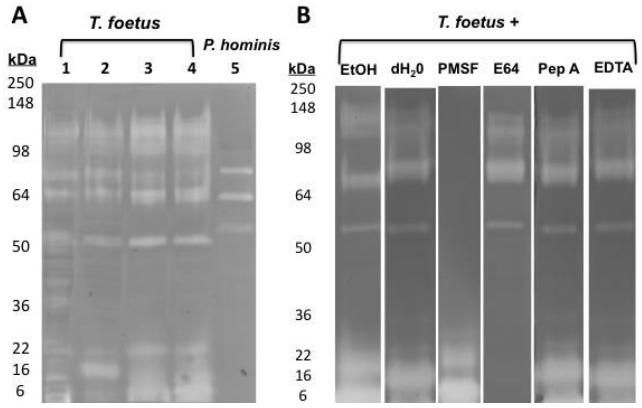

Figure 1. (A) Representative *In gel* zymography of cellular protein lysates from feline trichomonad isolates. Lanes 1-4, Tf isolates from 4 domestic cats; Lane 5, feline *P. hominis*. (B) The effect of inhibitors on protease activity of Tf protein lysates. Lane 1-2, no inhibitors (vehicle-treated controls); Lane 3, 5mM PMSF (serine protease inhibitor); Lane 4, 300μM E64 (CP inhibitor); Lane 5, 10μM pepstatin A (aspartic protease inhibitor); Lane 6, 5mM EDTA (metalloprotease inhibitor).[1]

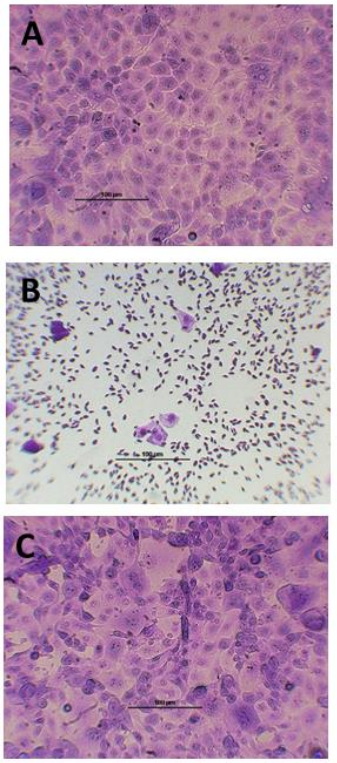

Figure 2. Inhibition of Tf CPs ameliorates Tf-induced cytotoxicity. Representative light microscopy images of crystal violet stained (A) uninfected porcine intestinal epithelial cell (IPEC) monolayers, (B) monolayers infected with vehicle-treated Tf or (C) CP inhibitor-treated Tf. After 24 hours of co-culture, few intestinal epithelial cells remain after infection with vehicle-treated Tf. Inhibition of Tf CP activity ameliorates cytopathogenicity in all Tf isolates tested (n= 4 isolates).

epithelial adhesion and induction of apoptosis by the venereal parasite.[8, 9] In a study of human women symptomatic for *T. vaginalis* infection, CP30 as well as antibodies against CP30 were detected in the serum of all 22 women tested.[10] Moreover, trichomonad isolates harvested from the symptomatic women had significantly more CP30 expression and higher cytoadherence to vaginal epithelial cells compared to isolates cultivated from asymptomatic *T. vaginalis*-infected women.[11] Together, these results suggest that CP30 plays an important role in the pathogenesis of venereal trichomonosis.

We have previously demonstrated that many virulence factors and mechanisms of cytopathogenicity are conserved between feline trichomonosis and venereal trichomonosis.[1, 12] Based on preliminary studies, we believe that CP30 is also conserved and expressed by feline Tf. Thus, CP30 represents a putative target for the prevention and/or treatment of feline trichomonosis. **In preliminary studies,** we have developed dot blot assays to evaluate for CP30 in feline Tf isolates. These early results suggest the presence of CP30 in feline Tf isolates **(Figure 3).**

Our objectives in these studies will be to characterize CP30 in six different feline Tf isolates and to determine the effect of CP30 inhibition on feline Tf CP activity and Tf-induced intestinal epithelial cell cytopathogenicity.

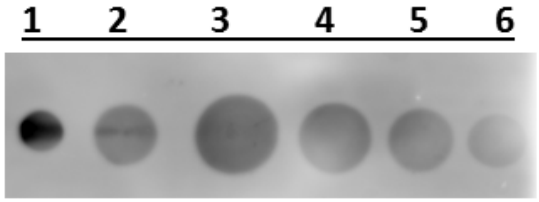

Figure 3. CP30 dot blot immunoassay. Representative dot blot of cellular protein lysates from feline Tf isolates for the presence of CP30. Lane 1, cattle Tf protein lysate, (positive control); Lanes 2-6, 80μg each of protein lysates from Tf isolates isolated from the feces of 5 different domestic cats.

Experimental Methods and Design: ***Feline Tf isolates:*** For these studies we will use 6 different isolates of feline Tf (to account for strain variability), 1 cattle *T. foetus* isolate (positive control known to express CP30) and one feline P. *hominis* isolate (negative control; presumptively nonpathogenic trichomonad control that has been demonstrated to lack CP activity, **Fig. 1** [1]) in each proposed experiment. Isolates of Tf and *P. hominis* isolates were harvested from the feces of naturally infected cats. Parasites will be cultivated at 37°C in modified Diamond's media with 10% inactivated equine serum and penicillin and streptomycin as previously described.[1] Only mid-log phase trichomonads with >95% viability as assessed by motility will be used for assays. ***Intestinal epithelial monolayers:*** In lieu of feline intestinal epithelial cell lines which do not currently exist, confluent monolayers of non-transformed porcine intestinal epithelial cells (IPEC-J2) cultivated on polystyrene plates will be used to model the intestinal epithelium as previously described.[12] Cultures with un-inoculated media (negative control) and Tf incubated with rabbit IgG (isotype control) will be used for all assays. Additional controls may be included as warranted. For all immunoassays we will use a polyclonal antibody directed against *T. vaginalis* CP30. This protein has been demonstrated to have high homology between *T. vaginalis* and bovine *T. foetus* and the antibody cross-reacts with bovine CP30. This antibody was kindly provided by Dr. Bibhuti Singh (Upstate Medical University).

(1) Immunoblot (Western blot) assays: To further characterize the presence of CP30 in 6 feline Tf isolates, we will harvest protein lysates from trichomonads ($20x10^6$ Tf) in mid-logarithmic-phase. Whole cell trichomonads will be lysed in radioimmunoprecipitation assay (RIPA) buffer, sonicated twice, and incubated for 30 min at 4°C. Supernatants containing protein lysate will be collected following pelleting of insoluble material at 15,800 x g for 10 min at 4°C. Protein lysate concentrations will be determined by BCA protein assay according to the manufacturer's instructions using bovine serum albumin (BSA) as standard. Lysates will be reduced, linearized, electrophoretically separated and transferred as previously described.[1] Following transfer, nitrocellulose membranes will be blocked overnight in blocking buffer (StartingBlock T20) at 4°C. The α-CP30 antibody diluted 1:1000 in 5% BSA will be applied and allowed to incubate for 4 h at RT. Following 3 washes with tris-buffered saline 0.05% Tween 20 (TBST) for 10 min each, the membrane will be incubated in HRP-conjugated goat anti-rabbit IgG diluted 1:5000 in 5% BSA for 30 min at RT. The membrane will be washed in TBST every 15 min for 2 h. A chemiluminescent agent will be applied for 5 min. Membranes will be developed with ImageQuant™ LAS 4000 software.

(2) Flow cytometry: For semi-quantitative analysis and localization of CP30 in feline whole organism trichomonads, flow cytometry will be utilized. Mid-log phase trichomonads (cultured for 48 h) will be centrifuged at 1500 rpm for 5 min, washed once in 1X PBS (pH 7.4) and re-suspended to 5 x 10^6 organisms/ml in flow buffer containing sodium azide. Isolates will then be divided into 1000 uL aliquots for the following samples: Tf receiving both primary and secondary antibody, Tf receiving only secondary antibody, rabbit IgG (negative controls) and Tf receiving no antibodies (autofluorescence). Tf will again undergo centrifugation. Isolates receiving primary antibody will be treated with α-CP30 antibody at a concentration of 1:500. All samples will be vortexed, incubated on ice for 30 min and undergo another wash in 1X PBS. All samples not denoted as autofluorescent will be incubated with FITC-conjugated F(ab)'2 goat anti-rabbit IgG at a concentration of 1:500, undergo a final wash and be re-suspended to 1000 uL in 1XPBS for analysis. Data will be acquired using the Attune Acoustic Focusing Cytometer with a minimum of 10,000 events counted per sample. A total of 2 feline and 1 bovine (positive control) isolates will be used for flow cytometric analysis, with 3 replicates performed per isolate. If isolates are negative for fluorescence without the addition of a permeabilizing agent (indicating intracellular localization), 4% PFA will substitute for flow buffer to allow intracellular penetration of antibodies.

(3) In gel zymography assay: To identify the effect of inhibition of CP30 on Tf CP activity, we will perform *In gel* zymography as we have previously described (**Fig. 1**).[1] To examine the effect of CP30 inhibition on Tf CP activity, we will incubate live Tf or protein lysate with α-CP30 antibody for 15 min at RT prior to protein extraction and/or electrophoresis. As controls, protein lysate will be run in the absence of antibody or will be treated identically with rabbit IgG (isotype control). Zones of proteolysis will be detected by the visualization of clear bands (areas where gelatin was digested through protease activity; see **Fig. 1** for example) against a stained background (areas where protease activity was not present). This assay will be performed at least 3 times to ensure that identical patterns of proteolysis are obtained with each sample.

(4) Crystal violet cytotoxicity assays: To provide a quantitative analysis of the effect of CP30 inhibition on amelioration of Tf-induced epithelial cytotoxicity, crystal violet (CV) assays will be performed as previously described [1] on uninfected monolayers and monolayers infected with isotype control-treated Tf or α-CP30 antibody treated Tf. CV stains the DNA of living cells. Therefore, cytotoxicity of intestinal epithelial monolayers stained with CV can be assessed qualitatively through visualization for loss of stain and quantitatively through spectrophotometric measurement of CV absorbance. IPEC-J2 monolayers will be cultivated to confluence on 24-well polystyrene plates prior to co-culture with 20 x 10^6 Tf for 24 hrs. Prior to co-culture, Tf will be treated with α-CP30 antibody or isotype control for 1 hr at RT. [22] After 24 hours of co-culture (time based on previously established model), the cells will be washed and stained with crystal violet as previously described.[1] Cells will then by solubilized in 1% sodium dodecyl sulfate in 50% ethanol and the staining intensity of the solubilized cells will be measured by a spectrophotometer (570nm wavelength). Assays will be performed in a minimum of 6 replicate cultures and repeated in triplicate experiments. Mean cytotoxicity will then be compared between experimental groups (Tf with or without MAb treatment) and control groups (no Tf).

Anticipated Results and Potential Pitfalls: Parasitic cellular proteases play critical roles in adhesion, cytopathogenicity and host immunoevasion. Therapies targeting parasitic proteases have already proven beneficial in eliminating disease or reducing morbidity in other protozoal and non-protozoal infections including Toxoplasmosis, Cryptosporidiosis, Leishmaniasis, Trypanosomiasis and venereal Trichomonosis.[5-8] We have previously demonstrated that CPs are important virulence factors for feline Tf. Singh and others have shown the importance of a specific CP, CP30, in mediating bovine and human trichomonad cytopathogenicity [8, 9]. Thus, we believe investigation into the presence and role of CP30 in feline Tf represents a promising new direction for treatment of feline trichomonosis. At the completion of these studies, we will have characterized the presence of CP30 in 6 different feline Tf isolates and evaluated the effect of inhibition of CP30 on feline Tf CP activity and intestinal cytopathogenicity. Porcine intestinal epithelial cells (IPEC-J2 cell line) will be used in lieu of feline intestinal epithelial cells for cytotoxicity studies since a feline intestinal cell line does not exist. However, we have previously validated that this co-culture model system mimics in vivo Tf epithelial cytopathogenicity.[1]

Data analysis: For all studies, a minimum of 6 replicates will be assessed for each isolate and antibody combination. Each study will be performed a minimum of 3 times. Raw data will be interpreted first for variance and normality followed by analysis with either parametric or non-parametric statistics where appropriate using SigmaStat (Jandell Scientific). Descriptive data will be generated from immunoblot and *In gel* zymography assays. Analysis of variance will be used to evaluate for differences between groups in cytotoxicity assays.

VI. Timeline

All experiments may be performed concurrently. Studies can be completed within one year (January 2016-January 2017).

Itemized Budget and Justification:

Immunoblot assays

Nitrocellulose membranes, BCA assay kit, LDS buffer, secondary antibody (goat anti-rabbit HRP IgG), Protease inhibitor kit, reducing agent, Bis-Tris gels, NuPage MES running buffer, transfer buffer, BSA, RIPA buffer, tris-buffered saline 0.05% Tween 20 (TBST), chemiluminescent substrate **$ 1704.00**

Flow cytometry assays

Fluorescent secondary antibody (goat anti-rabbit FITC F(ab')2 IgG, Jackson Immuno Research), Na Azide 0.5% w/v solution, 1X PBS, Fetal Bovine Serum **$ 716.00**

Substrate-Gel Electrophoresis Assays

RIPA buffer, BCA assay, LDS buffer, tris-glycine 0.1% gelatin gels, SDS (nondenaturating), running buffer, renaturing and developing buffers, colloidal blue (Various) **$ 1224.00**

Crystal violet assays and cell culture supplies		**$ 5452.21**
Media and additives		
Advanced DMEM/F12 (Fisher) $125.37 for 6-pack of 500 ml bottles x 5 packs	*$ 626.85*	
HBSS (w/o Ca, Mg; Fisher) $86.61 for 6-pack of 500ml bottles x 4 packs	*$ 346.44*	
dH_20 $80.70 for 6 pack of 500 ml bottles x 4 packs	*$ 322.80*	
Fetal Bovine Serum $256.45 for 500 ml x 3	*$ 769.35*	
Equine serum $98.60 for 500ml x 5	*$ 493.00*	
Insulin/Transferrin/Selenium (Fischer) $92.88 per 5 ml x 2 vials	*$ 185.76*	
Epidermal growth factor (BD) $155.40 for 100 µg x 2	*$ 310.80*	
Pen/Strep (Fisher) $31.75 for 100 ml x 6	*$ 190.50*	
Amphotericin (Fisher) case of 8 (20 ml)	*$ 283.71*	
Trichomonad culture additives: casein peptone, yeast extract, maltose, cysteine, L-ascorbic acid, penicillin	*$ 1585.00*	
Rabbit isotype control (Jackson Immunoresearch)	*$ 338.00*	
Culture disposables		**$ 3639.10**
Chamber slides 4 well pack @ $161.00 x 4	$ *644.00*	
Culture supplies: 15 and 50 cc conicals, sterile vials, serological pipettes, aspirating pipets, T75 and T25 tissue culture flasks, polystyrene plates	$ *1,007.50*	
Pipet tips $125.70, $125.70, $160.60 per case of 960 x 2 (Fisher)	$ *824.00*	
Media filter systems (250ml @ $104.30/case; 500 ml @ $186.60/case; Corning) x 4	$ *1163.60*	
Shipping charges		**$ 400.00**
Total supplies budget request		**$ 13135.31**
Salary request		**$ 8640.00**

Total budget (supplies and salary) request **$ 21775.31**

Personnel:

M. Katherine Tolbert (Role - Principal Investigator - 50% effort). Dr. Tolbert will be responsible for helping to conduct all assays. She will help with study design, data analysis, interpretation, presentation and preparation of the results for publication.

Mabre Brand (Role - Research Specialist - 50% effort). Ms. Brand will be involved with all aspects of this project including maintaining all trichomonad and epithelial cell cultures and carrying out all immunoassays and infection studies. We request **$6000 in salary and $2640.00 in fringe benefits** for Ms. Brand.***

***Note – The studies proposed in this grant application cannot be performed without considerable technical time commitment and expertise. Accordingly, we are requesting salary support for Ms. Brand. Her position in the laboratory is 100% soft money i.e. there are no state funds to support her technical commitment to this project.

Animal Justification Statement not applicable

References

1. Tolbert, M.K., et al., *Cysteine protease activity of feline Tritrichomonas foetus promotes adhesion-dependent cytotoxicity to intestinal epithelial cells.* Infect Immun, 2014. **82**(7): p. 2851-9.
2. Foster, D.M., et al., *Outcome of cats with diarrhea and Tritrichomonas foetus infection.* J Am Vet Med Assoc, 2004. **225**(6): p. 888-92.
3. Rosado, T.W., A. Specht, and S.L. Marks, *Neurotoxicosis in 4 cats receiving ronidazole.* J Vet Intern Med, 2007. **21**(2): p. 328-31.
4. Gookin, J.L., et al., *Documentation of in vivo and in vitro aerobic resistance of feline Tritrichomonas foetus isolates to ronidazole.* J Vet Intern Med, 2010. **24**(4): p. 1003-7.
5. Melendez-Lopez, S.G., et al., *Use of recombinant Entamoeba histolytica cysteine proteinase 1 to identify a potent inhibitor of amebic invasion in a human colonic model.* Eukaryot Cell, 2007. **6**(7): p. 1130-6.
6. Teo, C.F., et al., *Cysteine protease inhibitors block Toxoplasma gondii microneme secretion and cell invasion.* Antimicrob Agents Chemother, 2007. **51**(2): p. 679-88.
7. Cobo, E.R., S.L. Reed, and L.B. Corbeil, *Effect of vinyl sulfone inhibitors of cysteine proteinases on Tritrichomonas foetus infection.* Int J Antimicrob Agents, 2012. **39**(3): p. 259-62.
8. Singh, B.N., et al., *In vitro cytopathic effects of a cysteine protease of Tritrichomonas foetus on cultured bovine uterine epithelial cells.* Am J Vet Res, 2005. **66**(7): p. 1181-6.
9. Sommer, U., et al., *Identification of Trichomonas vaginalis cysteine proteases that induce apoptosis in human vaginal epithelial cells.* J Biol Chem, 2005. **280**(25): p. 23853-60.
10. Yadav, M., et al., *Cysteine proteinase 30 (CP30) and antibody response to CP30 in serum and vaginal washes of symptomatic and asymptomatic Trichomonas vaginalis-infected women.* Parasite Immunol, 2007. **29**(7): p. 359-65.
11. Yadav, M., et al., *Cysteine proteinase 30 in clinical isolates of T. vaginalis from symptomatic and asymptomatic infected women.* Exp Parasitol, 2007. **116**(4): p. 399-406.
12. Tolbert, M.K., S.H. Stauffer, and J.L. Gookin, *Feline Tritrichomonas foetus adhere to intestinal epithelium by receptor-ligand-dependent mechanisms.* Vet Parasitol, 2013. **192**(1-3): p. 75-82.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Tolbert, M. Katherine	Assistant Professor – University of Tennessee
Principal Investigator	Department of Small Animal Clinical Sciences 2407 River Dr. Knoxville, TN

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Berry College, Rome, GA	B.S.	2001	Animal Science
University of Georgia	D.V.M.	2006	Veterinary Medicine
North Carolina State University	Ph.D.	2013	Comparative Biomedical Sci (Major)/Biotechnology (Minor)

A. Positions and Honors.

2015 National Manuscript Clinical Sciences Award (mentoring author). Phi Zeta
2014 Young Investigator Award (3rd place). Am Vet Med Assoc & Am Vet Med Foundation.
2012 Best Oral Abstract Presentation ACVIM Forum, New Orleans, LA, (Comp Gastro. Society)
2011 Graduate Student of the Month (North Carolina State University)
2011 Comparative Gastroenterology Society Professional Development Award
2011 College of Veterinary Medicine Forum Presentation Winner (North Carolina State University)
2010-13 Graduate Student, (Dr. Jody Gookin, North Carolina State University, Comp Biomedical Sci)
2010 Best Oral Abstract Presentation ACVIM Forum, Anaheim, CA, (Comp Gastro Society)
2007-10 Resident in Small Animal Medicine (North Carolina State University
2006-7 Intern in Small Animal Medicine and Surgery, (UGA College of Veterinary Medicine)
2006 Forehand Clinical Excellence Veterinary Scholarship (UGA College of Veterinary Medicine)
2006 Cum Laude Graduate (UGA College of Veterinary Medicine)
2002 Research Specialist, (Dr. Amy Lee, Emory University, Neuropharmacology Department
2001 Magna Cum Laude Graduate (Berry College)
2001 Who's Who Recipient (Berry College)
2000,1 TranSouth Athletic Conference Scholar Athlete (Berry College)
2000,1 NAIA Academic All-American (Berry College)
2001 Omicron Delta Kappa Honoree (Berry College)
2001 Alpha Zeta Honoree (Berry College)

Memberships

American Gastroenterological Association
Comparative Gastroenterology Society
American College of Veterinary Internal Medicine

B. Selected Peer-Reviewed Publications and Abstracts (current and most relevant to study proposal; others provided on request)

1. **Tolbert MK**, Odunayo A, Howell R, Peters E, Reed A. Efficacy of Intravenous Administration of Combined Acid Suppressants in Healthy Dogs. J Vet Intern Med. 2015;29:556-60.
2. Parkinson S, **Tolbert K**, Messenger K, Odunayo A, Brand M, Davidson G, Peters E, Reed A, Papich M. Evaluation of the effect of orally administered acid suppressants on intragastric pH in cats. *J Vet Intern Med*. 2015;29(1):104-12.
3. **Tolbert MK,** Stauffer SH, Gookin JL. CPs mediate feline *Tritrichomonas foetus* adhesion-dependent cytopathogenicity. *Infect and Immun* 2014;82(7):2851-2859.
4. **Tolbert MK,** Stauffer SH, Gookin JL. Feline *Tritrichomonas foetus* adhere to intestinal epithelium by receptor-ligand-dependent mechanisms. *Vet Parasitol*. 2013;192(1-3):75-82.
5. **Tolbert MK,** Leutenegger CM, Lobetti R, Birrell J, Gookin J. Species identification of trichomonads and associated coinfections in dogs with diarrhea and suspected trichomonosis. *Vet Parasitol*. 2011;187(1-2):319-22.
6. **Tolbert K,** Bissett S, King A, Davidson G, Papich M, Peters E, Degernes L. Efficacy of oral famotidine and two omeprazole formulations for the control of intragastric pH in dogs. *J Vet Int Med*. 2011;25(1):47-54.
7. **Tolbert K** and Gookin JL. *Tritrichomonas foetus*: A new agent of feline diarrhea. *Compend Contin Educ Vet*. 2009;31(8):374-81.

8. Carleton, RE and **Tolbert MK** Prevalence of *Dirofilaria* immitis and gastrointestinal helminths in cats euthanized at animal control agencies in northwest Georgia. *Vet Parasitol*. 2004;119(4):319-326

Abstracts from Scientific Conferences (* indicates presenter)

1. **Tolbert K***, Gould E, Brand M. Feline *T. foetus* cytotoxicity can be inhibited by selective, small-molecule CP inhibitors. *33rd ACVIM Annual Forum.* Indianapolis, Indiana. June 2015. 2015;29(4).
2. Gould E*, Brand M. **Tolbert K** (mentoring author). Qualitative and quantitative identification of feline *Tritrichomonas foetus* surface antigens: Putative targets for diagnosis and treatment. *33rd ACVIM Annual Forum. J Vet Intern Med* 2015;29(4).
3. **Tolbert MK*.** Rational selection and use of acid suppressants. *33rd ACVIM Annual Forum*.
4. **Tolbert K***, Gould E, Brand M. The use of select CP inhibitors to prevent T. foetus cytopathogenicity. GutSea. Belize. 2015.
5. Parkinson S*, **Tolbert K**, Messenger et al. Efficacy of oral acid suppressants in cats. GutSea. Belize. 2015.
6. Howell R*, **Tolbert K**, Odunayo A. Evaluation of the efficacy of simultaneous acid suppressant therapy in dogs. IVECCS Forum. 2014. Indianapolis, IN.
7. Parkinson S, **Tolbert K***, Messenger K, et al. Evaluation of the efficacy of oral acid suppressants in cats. 32nd Annual Forum of the ACVIM. *J Vet Intern Med*. 2013;27(3). Nashville, TN.
8. **Tolbert MK***, Stauffer SH, Gookin JL. Serine and CPs of feline *T. foetus* promote survival and adhesion to intestinal epithelial cells. *31st Annual Forum of the ACVIM*, Seattle, WA.
9. **Tolbert MK**, Stauffer S, Gookin J*. CPs of the enteric trichomonad *Tritrichomonas foetus* mediate adhesion to intestinal epithelial cells and enterocyte apoptosis. *Gastroenterology* 2013. *Amer Gastroenterology Association-Digestive Disease Week,* Orlando, Fl.
10. **Tolbert MK***, Stauffer SH, Gookin JL. CPs of feline Tritrichomonas foetus mediate adhesion-dependent cytotoxicity to intestinal epithelial cells. Comparative & Experimental Medicine and Public Health Research Symposium, Knoxville, TN. 2013.
11. **Tolbert MK***, Gookin JL. "Exogenous sialic acid and CP inhibition block adherence of Tritrichomonas foetus to the intestinal epithlium." *J Vet Intern Med* 2012;26(3). *30th ACVIM Annual Forum*. New Orleans, LA.
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3. **Companion Animal Fund (CAF), Tolbert MK (Co-PI),** Olin S, MacLane Reed A. How much does it burn? A pilot study evaluation of gastric acidity in cats with CKD"-$3417 (2015-2016).

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