2015 Grant Proposal for the Winn Feline Foundation

1. Title of Study:

Study Name: Identification and investigation of feline *Tritrichomonas foetus* surface antigens as a target for diagnosis and treatment

2. List ALL Principal Investigator(s) Information:

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

Institution: The University of Tennessee College of Veterinary Medicine

Email:

Mailing Address:

b. Name: Emily N. Gould, DVM, MS candidate (Comparative & Experimental Medicine)

Institution: The University of Tennessee College of Veterinary Medicine

Email:

Mailing Address:

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

Agency Name:

Mailing Address:

EIN Number (US Applicants):

Check Made Payable to:

Grant Administrator Name:

Grant Administrator Email:

4. Amount Requested:

$ 16,077.76**.**

Signatures:

Signature of the principal investigator and appropriate grant administrator:

Signature: *Katherine Tolbert*

Signature: *Jane Burns*

Typing your name above constitutes electronic signature.

II. Scientific Summary Abstract

Tritrichomonas foetus (Tf) is a flagellated protozoal parasite that infects the distal ileum and proximal colon of domestic cats. Tf is a prevalent cause of severe, chronic diarrhea in cats worldwide. Only one drug is available to treat feline Tf and this drug is associated with increasing resistance and the risk of neurotoxicity. Tf infection also induces reproductive failure in cattle. Despite a difference in organ tropism between Tf genotypes, our lab and others have shown evidence for conserved virulence factors between feline and bovine Tf. Two surface antigens (1.15, 1.17) on bovine Tf participate in adhesion and cytotoxicity towards the urogenital epithelium. Inhibition of these antigens ameliorates bovine Tf-induced cytotoxicity. We have demonstrated that cytotoxicity of feline Tf is dependent upon adhesion to the intestinal epithelium. In preliminary studies, we identified the presence of surface antigen 1.15 on an isolate of feline Tf. Thus, identification of 1.15, 1.17 surface antigens on all feline Tf isolates and inhibition of adhesion by antibody opsonization may result in the identification of novel diagnostic and treatment strategies, respectively, for feline trichomonosis. The aims of the proposed study are: 1) to determine if all feline TF isolates tested express surface antigens 1.15 and 1.17 using indirect enzyme-linked immunosorbent assay and immunoblot studies, and 2) to evaluate the role of these antigens in mediating feline Tf intestinal cytopathogenicity using a validated co-culture model system. We believe the results of these studies will identify novel targets for development of diagnostic and/or therapeutic strategies for feline trichomonosis.

III. Lay-language abstract

Tritrichomonas foetus (Tf) is a protozoal parasite that is a prevalent cause of chronic diarrhea in domestic cats globally. The reported prevalence rate is as high as 30% in densely housed environments. No rapid, bedside assays are available to diagnose this infection. Moreover, only one drug is available to treat feline Tf and this drug is associated with increasing treatment failure and unacceptable side effects. As feline Tf closely resembles other intestinal infections of cats, it can be challenging for veterinarians to rapidly diagnose and treat Tf infection. Tf also causes abortions in cattle. Our group has previously demonstrated that cat and cattle Tf share common strategies for infecting their hosts. Two surface markers (1.15, 1.17) on bovine Tf participate in establishment of infection and induction of clinical signs. Thus, the aims of this study are to evaluate the expression of 1.15 and 1.17 in feline Tf and to determine if these markers play a role in injury of the intestine. All of our investigations can be performed on the benchtop using feline Tf organisms and a previously validated co-culture model system. Therefore, no cats will be harmed as a result of these studies and no live animal studies will be necessary. A vaccine against 1.17 is commercially available and effective against bovine Tf. Therefore, the results of these studies may lead to the development of a novel therapy and/or diagnostic strategy for cats infected with feline Tf.

IV. No continuation study

V. Study Proposal

BACKGROUND AND SIGNIFICANCE: *Tritrichomonas foetus* (Tf) is a protozoal pathogen responsible for chronic, large bowel diarrhea in domestic felids and abortions and early embryonic death in cattle. Feline Tf is highly prevalent and globally distributed with infections recognized in North America, Europe and Australia among others. [2-7] Feline trichomonosis presents a preventative, therapeutic, and diagnostic challenge to veterinarians. **Firstly, there are no vaccines for prevention of feline trichomonosis.** The only strategy for minimizing spread of Tf infection is to reduce close crowding in cats with high suspicion of infection. Although prolonged periods of clinical remission may occur, cats may remain subclinical, chronic carriers resulting in continued spread of the pathogen. [8] Thus, shelters and other high density housing environments may not recognize an infected cat before an outbreak occurs. **The development of a vaccine would decrease the prevalence of disease in susceptible cats.**

Secondly, there are limited effective and safe treatment strategies for feline Tf infection. [9,10] Ronidazole, the only treatment identified to be effective in some cats with trichomonosis, has been associated with neurotoxicity and development of drug resistance. [11-14] Thus, there is a compelling need for the development of novel therapies for the treatment of feline trichomonosis.

Thirdly, identification of feline Tf infection can also prove to be a diagnostic challenge. Direct smear light microscopy is the most widely available and inexpensive assay for the diagnosis of Tf. However, direct smear has a low sensitivity (positive in only 14% of naturally infected cats [15]) and misdiagnosis may be common given that feline Tf is often present as a co-infection with *Giardia*. [3, 15-17] Misdiagnosis can result in delay of therapy and/or risk of side effects in cats who receive inappropriate treatment. The In-Pouch™ TF-Feline test (Bio-Med Diagnostics) has an improved sensitivity (50%) compared to light microscopy [18], however, like light microscopy, pouch culture requires viable trichomonads (cannot use refrigerated fecal samples) and can result in bacterial overgrowth if too much fecal matter is applied to the culture. False positives in the presence of other trichomonads (e.g. *Pentatrichomonas hominis)* may also result in misdiagnosis. [19] PCR is the gold standard assay for diagnosis of feline Tf [20], however results may take up to 1-2 weeks and may not be a viable financial option for some owners and shelters. **Thus, the development of a rapid and inexpensive bedside assay would facilitate early diagnosis and treatment.**

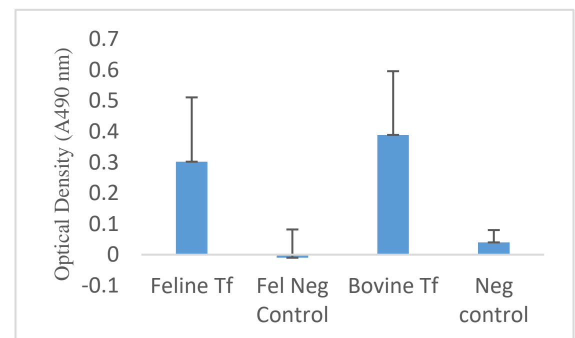

Figure 1. Presence of surface antigen 1.15 in feline Tf isolate confirmed via indirect ELISA. Modified from Ikeda et al [1], a total of three different organism concentrations were evaluated (5 x 10^4, 10^5, and 10^6 organisms/100 ul). 5 x 10^4 organisms/100 ul (per well) was determined to be an optimal antigen concentration. The remainder of ELISA optimization was performed to identify an ideal blocking agent (PBS-TW-G) and incubation time (1 hr in 37°C). Feline Tf (10.29.14) demonstrated 1.15 positivity as compared to positive Bovine Tf (11.5.14) and negative controls.

Bovine Tf infects the reproductive tract in cattle, however despite this difference in tropism, bovine and feline Tf are genetically similar and utilize comparable virulence factors in their pathogenesis [21-24] We demonstrated that feline Tf, just like bovine Tf, express cysteine proteases that promote adhesion-dependent cytotoxicity towards the intestinal epithelium. [22] However, inhibition of Tf cysteine protease activity does not result in complete inhibition of epithelial injury. Thus, as would be expected with any eukaryotic parasite, additional virulence factors likely play a role. We believe that feline Tf shares additional mechanisms of pathogenicity with bovine Tf. **Our lab is dedicated to the identification of bovine Tf virulence factors that are shared by feline Tf, and therefore serve as novel targets for the development of preventative, treatment and diagnostic strategies in feline trichomonosis.**

We have demonstrated, similar to bovine Tf, that cytotoxicity of feline Tf depends on adhesion to the intestinal epithelium. [22] Thus, feline Tf surface antigens may serve as the perfect target for the development of a diagnostic and treatment strategy. Hodgson et al [25] identified two surface antigens of bovine Tf (1.15, 1.17) that when inhibited by monoclonal antibodies (MAbs 1.15, 1.17) significantly inhibited trichomonad adhesion to bovine vaginal epithelium. Later studies demonstrated conservation of both antigens amongst 37 bovine isolates. [1] Tf1.17 (also known as lipophosphoglycan (LPG) complex protein [26]), is recognized as a major surface antigen with expression of 10^6 1.17/LPG complex/bovine Tf organism. [27] Heifers vaccinated with Tf1.17 developed a robust local immunoglobulin response [28], cleared infections more rapidly [29-32], and had decreased severity of endometritis as assessed by histopathology than non-vaccinated cattle. [29] Additionally, vaccination of bulls with Tf1.17 protected against trichomonad colonization of preputial and penile epithelium. [33] Inhibition of adhesion by antibody opsonization of surface antigens such as 1.15 and 1.17 may result in prevention of infection and/or amelioration of clinical signs in cats with trichomonosis. Based on preliminary

studies, we believe that one or both of these bovine Tf surface proteins may be conserved in feline Tf isolates and thus would represent a novel target for the development of a diagnostic and/or treatment strategy.

Research Hypothesis and Specific Aims

We hypothesize that the expression of bovine Tf surface antigens 1.15 and 1.17 are conserved in feline Tf and therefore one or both of these antigens will serve as novel targets for the development of novel preventative, treatment and/or diagnostic strategies for feline trichomonosis.

The **specific aims** of this proposal are 1) to identify if bovine Tf surface antigens 1.15 and/or 1.17 are present on all feline Tf isolates tested; and, 2) to evaluate the role of surface antigens 1.15 and 1.17 in feline Tf intestinal cytopathogenicity.

In preliminary studies, we have developed indirect enzyme-linked immunosorbent and dot blot assays to evaluate for the presence of surface antigens 1.15, 1.17 in feline Tf isolates. Early results suggest the presence of at least one of these antigens (1.15) on the surface of feline Tf **(Figures 1 and 2).**

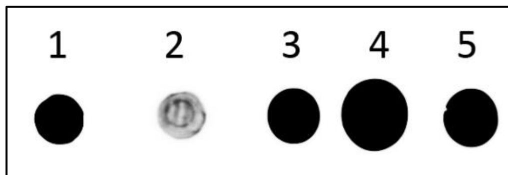

Figure 2. Presence of bovine Tf surface antigen 1.15 on feline Tf isolates. Representative dot blot of cellular protein lysates from feline trichomonad isolates for the presence of surface antigen 1.15. Lane 1, Positive control (1.15 antibody); Lane 2, Negative control (40µg IPEC-J2 protein lysate); Lanes 3-5, 40µg each of protein lysates from Tf isolates from 3 different domestic cats.

EXPERIMENTAL DESIGN: ***Feline Tf isolates:*** For all studies we will use 6 isolates of feline Tf (to account for strain variability) collected from fecal samples obtained from naturally infected cats geographically distributed throughout the USA. An isolate of feline *Pentatrichomonas hominis* (Ph) and bovine Tf will be used in all assays as negative and positive controls, respectively. All isolates will be cultivated as previously described. [34] ***Intestinal epithelial monolayers:*** To model the intestinal epithelium, we will use non-transformed cultures of porcine intestinal epithelial cells (IPEC-J2) that will be seeded on polystyrene plates and allowed to reach confluence before infection. IPEC-J2 will be used for all studies pending the development of a feline intestinal epithelial cell line. Cultures with un-inoculated media (negative control) and Tf incubated with antibody diluent (isotype control) will be used for all assays. Additional controls may be included as warranted. For all immunoassays we will use monoclonal antibodies (MAbs) targeting bovine surface antigens 1.15 and 1.17 that were kindly provided by Dr. Bibhuti Singh (Upstate Medical University).
***(1) Enzyme-linked immunosorbent assay (ELISA)*:** An indirect ELISA will be performed as previously described with some modifications. [1, 25] Tf will be harvested at mid-logarithmic phase, centrifuged at 15000 rpm for 5 minutes and suspended in 5% formalin in phosphate buffered saline (PBS pH 7.4) at 10^7 organisms/ml. Isolates will be washed in PBS prior to use. Organisms will be fixed to 96 well microtiter plates (Fisher Scientific) at 5 x 10^4/well and allowed to adhere overnight at room temperature (RT). Wells will be washed using a plate washer (ELx405, Bio-Tek Instruments, Inc) and blocked with 100 µl PBS with 0.05% Tween 20 and 2% Gelatin (PBS-TW-G) for 1 hr at 37°C. Plates will be washed with PBS and 0.05% Tween 20 (PBS-TW). 100 µl of MAbs Tf1.15 or Tf1.17 diluted to 1:500 in 2% milk will be applied wells. Plates will be incubated for 1 hr at 37°C and washed with PBS-TW. 100 µl of HRP-conjugated goat anti-mouse IgG and IgM (Kirkegaard and Perry Laboratories Inc) diluted to 1:500 will be applied wells, incubated for 1 hr at 37°C and washed with PBS-TW. 100 µl of TMB HRP substrate (ThermoScientific) will be applied and incubated at 37°C until maximal color change is perceived. The plate will be read on an ELISA reader (ELx800, Bio-Tek) using Gen5 Data Analysis Software (Bio-Tek) at 490 nm wavelength with blanked and negative control samples. Blanked samples, containing no antigen to correct for plate background, and samples lacking primary antibody to determine cut off points, will be used in all assays.
(2) Indirect immunofluorescence: Immunofluorescence will be used to qualitatively evaluate for the presence of 1.15 and 1.17 on feline Tf isolates. Trichomonads will be centrifuged at 1500 rpm for 5 minutes, washed in PBS and fixed with 4% paraformaldehyde for 20 minutes at 4°C. Cells will be washed in PBS and applied to Plus slides (Fisher Scientific) at 5 x 10^6 Tf/ml using a Cytospin (Shandon Cytospin® 2 Centrifuge). Slides will be rinsed briefly in PBSplus and permeabilized with Triton X-100 (Sigma Aldrich) for 10 minutes at RT. Slides will be rinsed with PBS prior to 1 hr incubation in block buffer (PBS Plus, 5% goat serum, 2% BSA). Slides will be incubated in primary MAb (1.15 or 1.17) diluted 1:100 in block for 3 hours in a humidified chamber. Following incubation, slides will be rinsed thrice in PBS for 5 minutes each. Cy5 goat anti-mouse IgG and IgM (Jackson Immuno Research) diluted 1:500 in block will then be applied and incubated for 1 hour at RT. Slides will be washed thrice with PBS for 5 minutes each. A DAPI counterstain (Vectashield, Vector

Laboratories) will be applied for visualization of the nucleus. A mercury camera will be used for detection of immunofluorescence.
(3) Immunodot analysis (Dot Blot) Dot blot analysis will be used as an additional qualitative assay to evaluate for the presence of 1.15 and 1.17 in 6 feline Tf isolates. Protein lysate will be used for all assays. This lysate will include all cellular protein (not just membrane-bound), and therefore will be able to identify antigens if they are not readily accessible on the cell surface (which may lead to a false negative with immunofluorescence if permeabilization is not effective in permitting access to these proteins). Protein concentration determination will be performed using albumin as a standard (BCA Assay Kit, Thermo Scientific). 40 µg of each protein lysate will be applied to the center of each grid on a nitrocellulose membrane. Protein lysate of IPEC-J2 cells will serve as a negative control. The membrane will be blocked with Starting Block™ T20 Buffer (Thermo Scientific) for 1 hr at RT. MAb 1.15 or 1.17 diluted 1:500 in tris-buffered saline and 0.05% Tween 20 (TBST) will be applied and allowed to incubate for 4 hrs at RT. Following 3 washes with TBST for 10 minutes each, the membrane will incubate in goat anti-mouse IgG and IgM diluted at 1:500 in TBST for 30 minutes at room temperature. The membrane will be washed in TBST 6 times for 1 hr. A chemiluminescent agent (Thermo Scientific) will be applied for 5 minutes. Membranes will be developed with ImageQuant™ LAS 4000 software.
(4) Crystal violet cytotoxicity assays: To provide a quantitative analysis of the effect of MAbs 1.15 and 1.17 on amelioration of Tf-induced epithelial cytotoxicity, crystal violet (CV) assays will be performed on uninfected monolayers and monolayers infected with isotype control-treated Tf or MAb (1.15, 1.17)-treated Tf. CV stains 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 36 hrs. Prior to co-culture, trichomonads will be treated with MAbs or vehicle for 1 hr at RT. [22] After 24 hours of co-culture (time based on previously established model), the cells will be washed twice with 37°C PBS and fixed with 2% paraformaldehyde in PBS for 15 minutes at RT. Cells will be washed with PBS and stained with 0.13% CV in 5:2 (v/v) ethanol-paraformaldehyde for 5 minutes. Cells will be washed twice with dH_20 and allowed to air dry. Wells will be evaluated for % of monolayer destruction (light microscopy) and compared between groups. 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. The degree of cytotoxicity will be expressed via the following formula: Cytotoxicity= 1- (measured $\text{intensity}_{\text{experimental}}$/measured $\text{intensity}_{\text{control}}$). 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: Upon completion of these assays, we anticipate to have identified at least one bovine Tf surface antigen shared by feline Tf isolates that will serve as a novel target for the development of innovative preventative, therapeutic and/or diagnostic strategies. Given that previous studies in bovine Tf led to development of a vaccine successful in ameliorating clinical signs of in cattle, we feel results from the proposed studies may lead to development of a feline vaccine. The ideal model for these studies would be feline intestinal epithelial cells, however this cell line does not exist. Therefore, these studies will utilize porcine intestinal epithelial cells (IPEC). However, the IPEC-J2 cell line is a suitable alternative as the porcine trichomonad, *Tritrichomonas suis,* is genetically similar to Tf and exerts the same tropism for the gastrointestinal tract. Moreover, we have validated that this co-culture model system mimics in vivo Tf epithelial cytopathogenicity. [22, 34]

DATA ANALYSIS: For all studies, a minimum of 6 replicates will be assessed for each isolate and antibody combination. Each study will be performed three times. For ELISA, the average of blanked samples will be subtracted from each reading to account for background, and a positive will be defined as two standard deviations above the mean of each negative control. For each isolate, outliers will be defined as values two standard deviations above or below the mean of the sample. Raw data will be interpreted first for variance and normality, as well as analyzed with either parametric or non-parametric statistics where appropriate using SigmaStat (Jandell Scientific). Descriptive data will be generated from immunodot analysis and indirect immunofluorescence assays. Analysis of variance will be used to evaluate for differences between groups in cytotoxicity assays.

VI. Timeline
All specific aims may be performed concurrently. Studies can be completed within one year.

VII. Itemized Budget:

Enzyme -Linked Immunosorbent Assays

Gelatin block buffer, PBS buffer, 96 well flat bottom plates, secondary antibody (goat anti-mouse HRP IgG+IgM), TMB Substrate, multi-channel pipette tips $ 1452.00

Immunofluorescence Assays

Fluorescent secondary antibody (goat anti-mouse HRP IgG+IgM, Jackson Immuno Research), goat serum (Jackson Immuno Research), Triton X-100, CaCl2 dihydrate, cover slips, coplin jars, Vectashield with DAPI (Vector Laboratories), pap pens, PLUS slides $ 716.00

Dot blot assays

Nitrocellulose membranes, BCA assay kit, secondary antibody (goat anti-mouse HRP IgG+IgM), tris-buffered saline 0.05% Tween 20 (TBST), Starting block buffer (Thermo Scientific), chemiluminescent substrate $ 600.00

Crystal violet assays and cell culture supplies

Item		Cost
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 4	$ 1025.80
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
Culture disposables		
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, transwell polycarbonate plates, polystyrene plates		$ 3,407.50
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
Misc – Crystal violet, PFA, ethanol, methanol PBS, SDS, gloves, paper supplies		$ 1500.00
Shipping charges		$ 400.00
Total budget request		**$** 16,077.76

Personnel:

M. Katherine Tolbert Role: Principal Investigator 25% effort
Dr. Tolbert will be responsible for helping to conduct all assays. She will help to direct the study design, data analysis, and interpretation and publication.

Emily Gould Role: Co-principal Investigator 75% effort
Dr. Gould, a master’s student under the direction of Dr. Tolbert, will be responsible for conducting all assays. These studies will serve as the backbone of her thesis project.

XIII. Animal Justification Statement not applicable

IX. References

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BIOGRAPHICAL SKETCH

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

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.

2014 Young Investigator Award. 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 Digestive Disease Week Travel Award Recipient
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)
2001 TranSouth Athletic Conference Scholar Athlete (Berry College)
2001 NAIA Academic All-America (Berry College)
2001 Omicron Delta Kappa Honoree (Berry College)
2001 Alpha Zeta Honoree (Berry College)
2000 TranSouth Athletic Conference Scholar Athlete (Berry College)
2000 NAIA Academic All-American (Berry College)

Memberships

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

B. Selected Peer-Reviewed Publications and Abstracts (most relevant to study proposal)

1. **Tolbert MK,** Stauffer SH, Gookin JL. Cysteine proteases mediate feline *Tritrichomonas foetus* adhesion-dependent cytopathogenicity. *Infect and Immun* 2014;82(7):2851-2859.
2. **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.
3. **Tolbert MK,** Leutenegger CM, Lobetti R, Birrell J, Gookin JL. Species identification of trichomonads and associated coinfections in dogs with diarrhea and suspected trichomonosis. *Vet Parasitol*. 2011;187(1-2):319-22.
4. **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.
5. **Tolbert K** and Gookin JL. *Tritrichomonas foetus*: A new agent of feline diarrhea. *Compend Contin Educ Vet*. 2009;31(8):374-81.
6. 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

<u>Abstracts from Scientific Conferences</u> (* indicates presenter)

1. Howell R, **Tolbert K**, Odunayo A. Evaluation of the efficacy of simultaneous acid suppressant therapy in dogs. IVECCS Forum. 2014. Indianapolis, IN.
2. Parkinson S, **Tolbert K***, Messenger K, et al. Evaluation of the efficacy of oral acid suppressants in cats. 32nd Annual Forum of the ACVIM. *JVIM* . 2013;27(3). Nashville, TN.
3. **Tolbert MK***, Stauffer SH, Gookin JL. Serine and cysteine proteases of feline *T. foetus* promote survival and adhesion to intestinal epithelial cells. *31st Annual Forum of the ACVIM*, Seattle, WA.
4. **Tolbert MK**, Stauffer S, Gookin J*. Cysteine proteases 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.
5. **Tolbert MK***, Stauffer SH, Gookin JL. Cysteine proteases of feline Tritrichomonas foetus mediate adhesion-dependent cytotoxicity to intestinal epithelial cells. Comparative & Experimental Medicine and Public Health Research Symposium, Knoxville, TN. May, 2013.
6. **Tolbert MK***, Gookin JL. "Exogenous sialic acid and cysteine protease inhibition block adherence of Tritrichomonas foetus to the intestinal epithlium." *J Vet Intern Med* 2012;26(3). *30th Annual Forum of the Amer College of Vet Intern Med*. New Orleans, LA.
7. **Tolbert MK***, Gookin JL. "*Tritrichomonas foetus* adheres to intestinal epithelium via receptor-ligand and cysteine protease dependent mechanisms." *Digestive Disease Week*. San Diego, CA.
8. **Tolbert MK***, Gookin JL. "The adherence characteristics of *Tritrichomonas foetus* to the intestinal epithelium." *Center for Gastrointestinal Biology and Disease Research Competition*. Raleigh, NC.
9. **Tolbert M*,** Leutenegger C, Lobetti R, Birrell J, Gookin J. "Species identity of trichomonads in feces of dogs with diarrhea and association with enteric coinfection." *J Vet Intern Med* 2011;25(3):690. *29th Annual Forum of the ACVIM*. Denver, CO.
10. **Tolbert MK***, Gookin JL. "An Intestinal Epithelial Cell Culture Model of *Tritrichomonas foetus* Cytopathogenicity." *NCSU College of Vet Med Research Forum*. Raleigh, NC.
11. **Tolbert MK***, Magness SM, Gookin JL "Modeling feline intestinal epithelial pathogen interactions in vitro." *Comparative Medicine and Translational Research Training Program (CMTRTP) symposium*. Raleigh, NC.
12. **Tolbert MK*,** Bissett S, King A, Davidson G, Papich M, Peters E, Degernes L "Efficacy of Oral Famotidine and 2 Omeprazole Formulations for the Control of Intragastric pH in Dogs." *J Vet Intern Med* 2010;24(3):722. *28th Annual Forum of the ACVIM.* Anaheim, CA.
13. **Tolbert MK*,** Carleton RE. "Survey of cats euthanized by animal control agencies in Northwest Georgia for Dirofilaria immitis and intestinal nematode parasites." *63rd Annual Meeting of the Assoc of Southeastern Biologists* (2002) 49 (No. 2): 187. App State Univ, Boone, NC.

C. Current Research Support

1. Morris Animal Foundation (MAF), **Tolbert MK (PI)** (D14FE-302) Cysteine Proteases: Novel Molecular Targets for Pharmacologic Control of Feline Trichomonosis-$48,322 (12/1/13-12/1/14).

2. Comparative Gastroenterology Society. Parkinson S, **Tolbert K (co-PI, mentoring author)**, et al. Evaluation of oral acid suppressants in cats. $8000.00 (2013-2014).

3. Center of Excellence in Livestock Diseases and Human Health (COE), Tolbert MK (PI). Modeling the intestinal epithelium through recapitulation of the intestinal stem cell niche-$**14,900.** (2013-2014).

BIOGRAPHICAL SKETCH

Name	Position Title
Gould, Emily Nissa	Comparative & Experimental Medicine Masters Student – University of Tennessee College of Veterinary Medicine
Occupation Doctor of Veterinary Medicine	2407 River Drive Knoxville, TN (916) 803-5976 engould@ucdavis.edu

EDUCATION/TRAINING

Institution/Location	Degree	Year(s)	Field of Study
University of Tennessee College of Veterinary Medicine, Knoxville, TN	M.S.	2016	Comparative & Experimental Medicine
University of California at Davis, Davis, CA	B.S.	2006	Animal Biology
University of California at Davis, Davis, CA	D.V.M.	2012	Veterinary Medicine

A. Positions and Honors

2013-14 Specialty Intern in Small Animal Internal Medicine (Animal Specialty & Emergency Center, Los Angeles, CA)

2012-13 Intern in Small Animal Medicine and Surgery (Veterinary Medical and Surgical Group, Ventura, CA)

2012 Doctorate of Veterinary Medicine (UC Davis College of Veterinary Medicine)

2012 Oliver Chocolate Chip Cookie Scholarship Recipient (UC Davis College of Veterinary Medicine)

2006 Research Assistant (Dr. Leslie Lyons, University of California at Davis, Center for Comparative Animal Health, Laboratory of Feline Genetics)

2006 Who's Who Recipient (University of California at Davis)

2006 High Honors Recipient, Bachelors of Science in Animal Biology (University of California at Davis)

Memberships

American Gastroenterological Association (AGA)
Comparative Gastroenterology Society (CGS)
American Veterinary Medical Association (AVMA)

B. Selected Peer-Reviewed Publications and Contributions to Projects

Peer Reviewed Publications

1. **Gould E.N.**, Brand M., Tolbert M.K. Qualitative and quantitative detection of a *Tritrichomonas foetus* surface antigen that mediates intestinal epithelial cytotoxicity (abstr.). DDW 2015; submitted.
2. Tolbert M.K., **Gould E.N.,** Brand M. Targeted inhibition of *Tritrichomonas foetus* cysteine proteases ameliorates intestinal epithelial cytopathogenicity (abstr.). DDW 2015; submitted.
3. **Gould E**., Brand M., Tolbert M.K. Quantitative and qualitative identification of *Tritrichomonas foetus* surface antigens: Putative targets for diagnosis and treatment (abstr.). ACVIM Forum 2015; submitted.
4. Tolbert M.K., **Gould E.N.**, Brand M. Feline *T. foetus* cytotoxicity can be inhibited by selective, small-molecule cysteine protease inhibitors (abstr.). ACVIM Forum 2015; submitted.
5. Whittemore J.C., **Gould E.**, Newman S. Gastrointestinal endoscopic mucosal lesion scores in healthy dogs receiving prednisone, aspirin and omeprazole (abstr.). J Vet Intern Med 2015; 28: submitted.
6. **E.N. Gould,** S.R. Trivedi, T.A. Cohen, J.Y. Kim. Emphysematous pyelonephritis in a domestic shorthair cat. *J Feline Med Surg.* Publication re-submitted for review. Departments of Surgery and Internal Medicine, Animal Specialty and Emergency Center, Los Angeles 90025.
7. **Gould EN,** Johnson LR, Traslavina RP, Mohr FC. Neuroendocrine tumour at the carina of a dog. *J Comp Pathol.* 2013 Aug-Oct;149(2-3):233-6.
8. Carolyn A. Erdman, **Emily N. Gould**, Leslie A. Lyons. *Chromosomal localization of an inherited craniofacial defect in cats*. Department of Population Health & Reproduction, School of Veterinary Medicine, University of California, Davis. Created linkage map as a part of senior practicum and NIH funded research project.

C. Didactic teaching Knoxville, TN

VMD 887 Feline medicine elective (1 hr)

Application Based Learning Exercise (ABLE; 1 hr)

D. Continuing Education Contributions Ventura, CA

"Nasal Aspergillosis: Case Report and Review of the Literature". E.N. Gould. Case report and review of the literature distributed to referring veterinary community in Ventura, CA. February 2013.