Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 154 (2008) 156–161
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

# Experimental infection of cats (*Felis catus*) with *Tritrichomonas foetus* isolated from cattle

Heather D. Stockdale [a,*], A. Ray Dillon [b], Joseph C. Newton [a], Richard C. Bird [a], Robert H. BonDurant [c], Patricia Deinnocentes [a], Sharron Barney [a], Jamie Bulter [a], Tracey Land [a], Jennifer A. Spencer [a], David S. Lindsay [d], Byron L. Blagburn [a]

[a] *166 Greene Hall, Department of Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL 36849, USA*
[b] *612 Hoerlein Hall, Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL 36849, USA*
[c] *Department of Population Health & Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*
[d] *Virginia Polytechnical Institute and State University, Department of Biomedical Science and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Center for Molecular Medicine and Infectious Diseases, 1410 Prices Fork Road, Blacksburg, VA 24061-0342, USA*

Received 9 October 2007; received in revised form 19 February 2008; accepted 21 February 2008

**Abstract**

*Tritrichomonas foetus* is recognized as the causative agent of venereal trichomoniasis in cattle. It is characterized by embryonic and early fetal death and post-coital pyometra, and feline trichomoniasis, manifest as chronic, large bowel diarrhea. Many of the infected cats are less than 2 years old and specific routes of transmission remain unknown. We recently demonstrated that feline isolates of *T. foetus* can successfully infect heifers, resulting in pathologic changes similar, but not identical to those previously reported as representative of bovine trichomoniasis. In this study, we experimentally infected six cats less than 1 year of age with a bovine (D-1) isolate of *T. foetus* and one cat with a feline (AUTf-1) isolate of *T. foetus*. Within 2 weeks, the cat infected with the feline (AUTf-1) isolate was culture positive for trichomonads in weekly fecal samples. At the end of 5 weeks, only one cat infected with the bovine (D-1) isolate was fecal culture positive for trichomonads. At necropsy, the intestine of each cat was removed and divided into five sections (ileum, cecum, anterior, medial and posterior colon). Contents from each section were collected and cultured. The cat infected with the feline (AUTf-1) isolate was culture positive in the ileum, cecum, medial and posterior colon. Two cats infected with the bovine (D-1) isolate were culture positive in the cecum only. Additionally, each intestinal section was submitted to a pathologist for histopathological examination. The combined results indicate that there are demonstrable differences between the feline (AUTf-1) and bovine (D-1) isolates regarding their infectivity in cats.


*Keywords:* *Tritrichomonas foetus*; Feline trichomoniasis; Experimental infection

## 1. Introduction

*Tritrichomonas foetus* is the causative agent of bovine trichomoniasis, a disease of the reproductive tract resulting in infertility and abortions in infected cows (BonDurant, 1985, 1997; Felleisen, 1999). Infected bulls become chronic carriers, while infected cows may clear the infection within 2–6 months (BonDurant, 1997; Stockdale et al., 2007). In addition, *T. foetus* has recently been recognized as an agent of feline large bowel disease (Foster et al., 2004; Gookin et al., 1999, 2001; Levy et al., 2003). Over the past decade, the numbers of reports of *T. foetus*-induced

* Corresponding author. Tel.: +1 334 844 2698; fax: +1 334 844 2652.
*E-mail address:* stockhd@auburn.edu (H.D. Stockdale).

0304-4017/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2008.02.024

large bowel diarrhea in cats has increased (Stockdale et al., 2006). Surveys of cats from the United States and other countries have demonstrated that *T. foetus* is found in both purebred and mixed breed cats that may or may not have been in contact with cattle (Gookin et al., 1999, 2004). Clinical signs of disease in infected cats include diarrhea with mucus, lethargy, anorexia and weight loss (Gookin et al., 1999, 2001; Jordan, 1956; Stockdale et al., 2006). Additional signs may include diarrhea with blood, tenesmus, flatulence and malodorous feces. Experimental infection of cats with feline-derived *T. foetus* has reproduced many of these signs (Gookin et al., 2001). In some reports, the diarrhea associated with trichomonad infection had been attributed to *Pentatrichomonas hominis* (Romatowski, 1996, 2000). However, it was later shown that *T. foetus* was the causative agent and not *P. hominis* (Levy et al., 2003).

Previous research has demonstrated that a feline isolate of *T. foetus* can successfully infect bovines. However, the resulting disease differs from that caused by a bovine isolate of *T. foetus* (Stockdale et al., 2007). To our knowledge, there exist no published reports of attempts to experimentally infect cats with a bovine isolate of *T. foetus*. In this study, we report the results of experimental infection of cats with the D-1 bovine isolate of *T. foetus*.

## 2. Materials and methods

Two isolates of *T. foetus*, a feline isolate (AUTf-1) collected from a naturally infected cat presented to Auburn University College of Veterinary Medicine (Stockdale et al., 2006) and a bovine isolate (D-1) originally collected from a naturally infected, pyometritic cow (Skirrow and BonDurant, 1990), were obtained and cultured in trypticase-yeast-maltose (TYM) media without agar (Diamond, 1983). These isolates were kept in freezing media in liquid nitrogen until 1 week before use. Freezing media consisted of fetal calf serum (FCS), dimethyl sulfoxide (DMSO) and TYM media at 3:2:5, respectively. They were then thawed and subcultured in TYM media at 37 °C.

Eight domestic shorthair cats, six females and two males, ranging in age from 8 to 12 months, were obtained from the Scott-Ritchey Research Center, Auburn University College of Veterinary Medicine. Fecal samples were collected from each cat to verify the absence of *T. foetus* by culture and polymerase chain reaction (PCR) (Grahn et al., 2005). Cats were housed in the same room in separate stainless steel cages. Lighting and temperature were automatically controlled and daily care and maintenance was provided by the Division of Laboratory Animal Health, Auburn University College of Veterinary Medicine. The cats were acclimated to the cages and environment for 10 days prior to experimental infection.

*Tritrichomonas foetus* DNA was isolated from fecal samples by first washing the feces three times with Tris–EDTA (TE) buffer (1 mM EDTA, 10 mM Tris, pH 8.0). After the final wash, the pellet was resuspended in 100 μl TE and DNA extraction for PCR analysis was carried out as described in Billeter et al. (2007). The PCR protocol, with modifications, described in Grahn et al. (2005) was used to identify *T. foetus* from culture and fecal samples by amplifying the internal transcribed spacer 1 (ITS1) region. Modifications included 1 mM 10× PCR buffer II, 1 mM $MgCl_2$, 0.2 mM dNTP and 0.05 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). Additionally, the annealing temperature was decreased to 56 °C for 30 s.

Cats were fasted for 24 h prior to inoculation. For experimental infection, cats were sedated using a mixture of medetomidine hydrochloride (Domitor®, Pfizer Animal Health, Exton, PA, USA) (68 mg/ml), butorphanol tartrate (Torbugesic®, Fort Dodge, KS, USA) (1.8 mg/ml) and ketamine (Ketaset®, Fort Dodge) (0.14 mg/ml) at a rate of 0.075 ml/kg. The AUTf-1 and D-1 isolates were washed and resuspended in antibiotic-free, fetal bovine serum-free TYM media without agar approximately 1 h prior to inoculation. Six cats received $1.5 \times 10^6$ trichomonads (D-1 isolate) in 10 ml of antibiotic-free, fetal bovine serum-free TYM media without agar via orogastric intubation. One cat was inoculated with $1.5 \times 10^6$ trichomonads (AUTf-1 isolate) in similar media. One cat was inoculated with media only. Cats were allowed to recover naturally.

Fecal samples were collected from each cat three times a week for 5 weeks. Samples were obtained per rectum using a plastic loop or from a freshly voided sample in the litter box. Each sample was immediately suspended in 2 ml of TYM media and incubated at 37 °C. In addition to sample collection, fecal consistency was noted using a scale of 1–4 (1 = diarrhea, watery, loose or possibly blood; 2 = no form, loose, puddles or piles; 3 = formed, soft or wet; 4 = formed and hard) (Purina® Fecal Scoring System for Cats, Nestlé Purina PetCare Co., St. Louis, MO, USA). Emesis or other signs were also noted. All samples were examined at 100× magnification after 2 days in culture. Samples were scored as positive (+) or negative (−) based on the presence of motile *T. foetus* trophozoites, and were verified using PCR (Grahn et al., 2005). Negative samples were held for 10 days and rechecked every 2 days.

At 5 weeks post-inoculation (PI), all cats were sedated as described above. Following sedation, the cats were euthanized by intravenous administration of sodium pentobarbital (Euthasol®, Delmarva Laboratories, Midlothian, VA, USA) at the dose of 17.7 mg/kg. At necropsy, the ileum, cecum and colon were removed as one section. The anterior ileum and posterior colon were ligated with cotton twine. Ligatures were also placed at the posterior ileum, base of the cecum and the anterior colon. The colon was then sub-divided into three equal sections using two additional ligatures. This yielded a total of five separate sections of bowel: terminal ileum, cecum, anterior, medial and posterior colon. Next, 2–5 ml of TYM media, depending on the size and fecal contents of the intestinal section, was injected into each section using a sterile 18-guage needle and 10 ml syringe. The sections were then incised and the contents of each section were collected into a separate specimen cup. One milliliter of contents collected from each section of intestine was resuspended in 5 ml of TYM media and incubated at 37 °C. Samples were examined as previously described for visible trophozoites and verified using PCR procedures (Grahn et al., 2005).

Tissues for histopathologic examination were fixed in 10% buffered formalin and stained with hematoxylin and eosin stain. Inflammation in the lamina propria of the ileum, cecum and colon was subjectively evaluated, in a blinded fashion, with light microscopic examination of tissue sections at 100× magnification. Inflammation was characterized based on the presence of lymphocytes and plasma cells and their distribution within the intestinal lamina propria. Each slide was scored using a scale of increasing severity: 1 = minimal, slight, 2 = moderate and 3 = severe, marked (Stockdale et al., 2007).

## 3. Results

Motile trichomonads were successfully observed in cultures from three fecal samples obtained from the positive control cat (cat no. 7), inoculated with the feline (AUTf-1) isolate, beginning day 16 PI (Table 1). Of the six cats inoculated with the bovine (D-1) isolate, only one cat (cat no. 2) was culture positive on day 32 PI (Table 1). The remaining five cats inoculated with the bovine (D-1) isolate remained culture negative throughout the study. Neither of the two cats (no. 2 bovine [D-1] isolate and no. 7 feline [AUTf-1] isolate) that were culture positive developed diarrhea or demonstrable vomiting, loss of appetite or fever during the study. On day 9 PI, one cat (cat no. 5) inoculated with the bovine (D-1) isolate had soft stool with small amounts or blood and mucus, and cat no. 4 had episodes of vomiting and a fever of 39.3 °C on day 21 PI (Table 1), but neither cat was culture positive for *T. foetus*.

Motile trichomonads were successfully observed in cultures from the intestinal contents of both fecal culture positive cats (see above) and all results were verified by PCR. Cat no. 2, bovine (D-1) isolate, was culture positive in the cecum ($1.75 \times 10^4$ trichomotrichomonads/ml). Cat no. 7, feline (AUTf-1) isolate, was culture positive in the ileum, cecum, medial colon and posterior colon ($1.73 \times 10^5$, $3.15 \times 10^6$, $2.5 \times 10^3$ and $5.0 \times 10^3$ trichomonads/ml, respectively). Additionally, cat no. 4 bovine (D-1) isolate was culture positive in the cecum ($2.75 \times 10^4$ trichomonads/ml). Cat no. 8 (media only), was culture positive in the ileum. However, this was an erroneous result due to improper labeling of tubes during subculture. That a negative diagnosis was appropriate for this culture is supported by the fact that PCR results in all samples obtained from the original specimen cups at necropsy,

Table 1
Results of the fecal sample cultures and health observations of cats over the 5-week study period

| Cat ID | Isolate | No. positive fecal cultures/total no. samples | Signs of disease |
|---|---|---|---|
| 1 | D-1 | 0/15 | None |
| 2 | D-1 | PI 32d[a]; 1/15 | None |
| 3 | D-1 | 0/15 | None |
| 4 | D-1 | 0/15 | PI 21d; fever (102.8 F) vomiting |
| 5 | D-1 | 0/15 | PI 9d; blood and mucus in loose stool; fecal score = 1 |
| 6 | D-1 | 0/15 | None |
| 7 | AUTf-1 | PI 16d[a]; 3/15 | None |
| 8 | Media only | 0/15 | None |

Cats were inoculated with either the bovine D-1 isolate (nos. 1–6) or feline AUTf-1 isolate (no. 7) of *Tritrichomonas foetus*. One cat (no. 8) was not inoculated with trichomonads (media only) and used as a negative control. Over the 5-week period, 15 samples were taken from each cat, beginning post-inoculation (PI) day 2 (2d). All cats had fecal scores ranging from 3 to 4 (formed and either soft and wet or hard) unless otherwise noted.

[a] Day of first positive culture sample.

Table 2
Histopathological analysis results of intestinal sections recovered from each cat at necropsy

| Bovine D-1 isolate | Ileum | Cecum | Anterior colon | Medial colon | Posterior colon | Mean score |
|---|---|---|---|---|---|---|
| 1 | 2 | 3+ | 2+ | 3+ | 2+ | 2.4 |
| 2 | 1–2 | 1 | 1–2+ | 2+ | 2+ | 1.6 |
| 3 | 2 | 2+ | 2+ | 1 | 2+ | 1.8 |
| 4 | 2 | 1+ | 1 | 1 | 1–2+ | 1.3 |
| 5 | 1 | 2+ | 2 | 2+ | 2–3 | 1.9 |
| 6 | 1–2 | 1+ | 1+ | 2–3+ | 3+ | 1.8 |
| Feline AUTf-1 isolate | | | | | | |
| 7 | 1–2 | 2+ | 2+ | 3+ | 2–3+ | 2.4 |
| Media only | | | | | | |
| 8 | 2 | 2 | 2+ | 2+ | 3+ | 2.2 |

Intestinal sections were stained with hematoxylin and eosin and the presence of lymphocytes and plasma cells were scored on a scale of increasing severity (1 = minimal, slight, 2 = moderate, 3 = severe, marked). Individual scores for each intestinal section recovered from each cat are shown along with overall mean scores.

and from weekly fecal samples obtained throughout the study, were negative.

Tissue samples were obtained and scored as described above from each intestinal section (Table 2). All intestinal samples taken from the cat inoculated with the feline (AUTf-1) isolate showed an increase of lymphocytes and plasma cells in the mucosa, which appeared to be migrating from moderately hyperplastic Peyer's patches (Fig. 1A). All samples taken from cats inoculated with the bovine (D-1) isolate demonstrated similar lesions (Fig. 1B). Each cat had some level of lymphocyte and plasma cell infiltration in the sections of intestinal mucosa, although some were more pronounced than others (Table 2). Samples collected from the non-infected control cat displayed similar levels of lymphocytes and plasma cells however Peyer's patches and surrounding mucosa appeared normal (Fig. 1C).

## 4. Discussion

Results of this study suggest that differences exist in both infectivity and pathogenicity for feline (AUTf-1) and bovine (D-1) isolates in experimentally infected cats. These results are similar to those of our previous study in which heifers infected with the AUTf-1 isolate of *T. foetus* demonstrated temporal differences in infection and severity of disease when compared to heifers infected with a bovine (D-1) isolate of *T. foetus* (Stockdale et al., 2007). Of the six cats inoculated with the bovine (D-1) isolate, only one was positive by fecal culture during the 5-week study. This occurred on the last day of sampling. The cat inoculated with the feline (AUTf-1) isolate was positive by fecal culture at 15 days PI. PCR results supported that negative fecal cultures were truly negative and not the result of too few organisms to culture successfully. Positive fecal cultures were also positive by PCR, supporting the sensitivity of the fecal culture method (Gookin et al., 1999, 2003, 2004; Grahn et al., 2005).

Similar results were obtained from culture samples from intestinal contents of the ileum, cecum and colon of each cat at necropsy. The cat inoculated with the feline (AUTf-1) isolate was again culture positive in the ileum, cecum, medial and distal colon. Of the six cats inoculated with the D-1 isolate, a single cat that was fecal culture positive was also positive when cecal contents were collected. One additional cat was culture positive when cecal contents were collected but was negative based on fecal culture. These results were again verified using PCR (Grahn et al., 2005).

It is possible that trichomonads are passed in feces intermittently or in low numbers only during episodes of diarrhea. Consequently, *in situ* sampling techniques would likely be more sensitive than fecal culture techniques. The use of PCR procedures for detection of trichomonads in organ contents or in feces would be expected to be more sensitive than culture, only requiring a small amount of DNA and able to easily distinguish between different trichomonads easily (Grahn et al., 2005). This is important when trichomonad populations are low or a mixed infection is suspected.

Interpretation of histologic changes in the colon can be difficult. Lymphocytes and plasma cells normally respond to antigens within the colonic lumen. Wide variation in numbers of lymphocytes and plasma cells is common place and can even be interpreted as a normal response to the ever-changing enteric microenvironment (Wilcock, 1992). Analysis of histopathologic

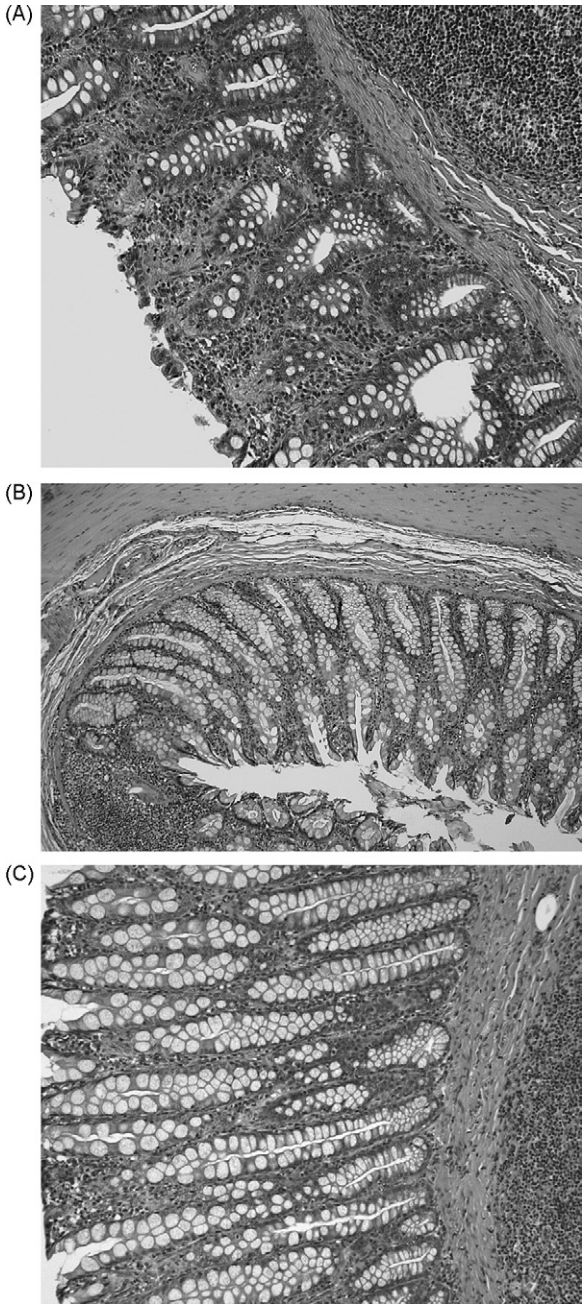


Fig. 1. Tissue samples taken at necropsy from the intestine of cats infected with *Tritrichomonas foetus*. The feline isolate, AUTf-1 (A), the bovine isolate, D-1 (B) and the non-infected control (C). Images are taken at 100× magnification.

changes in each feline intestinal section revealed aggregates of lymphocytes and plasma cells of approximately equal frequency and intensity in both treatment groups. Based on prior research (Yeager and Gookin, 2005), the experimental infection results suggest that longer infection times may be necessary to show demonstrably different pathogenic events in cats. These cats were not specific pathogen-free animals and the pathologic changes observed in the intestinal mucosa could be in response to any intraluminal antigen such as food antigens or antigens from one or several of the many species of bacteria and other microorganisms living in the colonic contents.

The results of this study together with the results of our previous research in bovines suggest that there are differences in biological or pathogenic behavior of the feline and bovine isolates that cannot be ignored. These data also suggest that direct transmission from bovines to felines is not the primary means of trichomonad infection in cats. The two studies provide compelling evidence that fundamental differences exist between the different isolates of *T. foetus*. In the opinion of the authors, these differences exceed what one would expect to observe in normal intra-specific variation.

## Acknowledgements

The authors would like to thank Drs. Henry Baker and Nancy Cox of the Scott-Ritchey Research Center, Auburn University College of Veterinary Medicine, for donating the cats used in this study.

## References

Billeter, S.A., Spencer, J.A., Griffin, B., Dykstra, C.C., Blagburn, B.L., 2007. Prevalence of *Anaplasma phagocytophilum* in domestic felines in the United States. Vet. Par 147, 194–198.

BonDurant, R.H., 1985. Diagnosis, treatment and control of bovine trichomoniasis. Compend. Contin. Educ. Pract. Vet. 7, S179–S188.

BonDurant, R.H., 1997. Pathogenesis, diagnosis, and management of trichomoniasis in cattle. Vet. Clin. North Am. Food Anim. Pract. 13, 345–361.

Diamond, L.S., 1983. In: Jensen, J.B. (Ed.), Lumen dwelling protozoa: *Entamoeba*, Trichomonads and *Giardia*. Boca Raton, Florida, pp. 65–111.

Felleisen, R.S.J., 1999. Host-parasite interaction in bovine infection with *Tritrichomonas foetus*. Microb. Infect. 1, 807–816.

Foster, D.M., Gookin, J.L., Poore, M.F., Stebbins, M.E., Levy, M.G., 2004. Outcome of cats with diarrhea and *Tritrichomonas foetus* infection. J. Am. Vet. Med. Assoc. 225, 888–892.

Gookin, J.L., Breitschwerdt, E.B., Levy, M.G., Gager, R.B., Benrud, J.G., 1999. Diarrhea associated with trichomonosis in cats. J. Am. Vet. Med. Assoc. 215, 1450–1454.

Gookin, J.L., Levy, M.G., Law, J.M., Papich, M.G., Poore, M.F., Breitschwerdt, E.B., 2001. Experimental infection of cats with *Tritrichomonas foetus*. Am. J. Vet. Res. 62, 1690–1697.

Gookin, J.L., Foster, D.M., Poore, M., Stebbins, M.E., Levy, M.G., 2003. Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats. J. Am. Vet. Med. Assoc. 222, 1376–1379.

Gookin, J.L., Stebbins, M.E., Hunt, E., Burlone, K., Fulton, M., Hochel, R., Talaat, M., Poore, M., Levy, M.G., 2004. Prevalence

of and risk factors for feline *Tritrichomonas foetus* and *Giardia* infection. J. Clin. Microbiol. 42, 2707–2710.

Grahn, R.A., BonDurant, R.H., Hoosear, K.A.v., Walker, R.L., Lyons, L.A., 2005. An improved molecular assay for *Tritrichomonas foetus*. Vet. Parasitol. 127, 33–41.

Jordan, H.E., 1956. *Trichomonas* spp. in feline: a case report. Vet. Med. 51, 23–24.

Levy, M.G., Gookin, J.L., Poore, M., Birkenheuer, A.J., Dykstra, M.J., Litaker, R.W., 2003. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline trichomonal diarrhea. J. Parasitol. 89, 99–104.

Romatowski, J., 1996. An uncommon protozoan parasite (*Pentatrichomonas hominis*) associated with colitis in three cats. Fel. Pract. 24, 10–14.

Romatowski, J., 2000. *Pentatrichomonas hominis* infection in four kittens. J. Am. Vet. Med. Assoc. 216, 1270–1272.

Skirrow, S.Z., BonDurant, R.H., 1990. Induced *Tritrichomonas foetus* infection in beef heifers. J. Am. Vet. Med. Assoc. 196, 885–889.

Stockdale, H.D., Spencer, J.A., Dykstra, C.C., West, G.S., Hankes, T., McMillan, K.L., Whitley, M., Blagburn, B.L., 2006. Feline Trichomoniasis: an emerging disease. Compend. Contin. Educ. Pract. Vet. 28, 463–471.

Stockdale, H.D., Rodning, S.P., Givens, M.D., Carpenter, D.M., Lenz, S.D., Spencer, J.A., Dykstra, C.C., Lindsay, D.S., Blagburn, B.L., 2007. Experimental infection of bovines with a feline isolate of *Tritrichomonas foetus*. J. Parasitol. 93, 1429–1434.

Wilcock, B., 1992. Endoscopic biopsy interpretation in canine or feline enterocolitis. Semin. Vet. Med. Surg. (Small Anim.) 7, 162–171.

Yeager, M.J., Gookin, J.L., 2005. Histologic features associated with *Tritrichomonas foetus*-induced colitis in domestic cats. Vet. Pathol. 42, 797–804.