

Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats

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Objective—To evaluate the efficacy of and optimize a commercially available culture system for sensitive and specific in-clinic culture of *Tritrichomonas foetus* from cat feces.

Design—Prospective study.

Sample Population—Samples of freshly voided feces from 117 purebred cats and pure cultures of *T foetus* obtained from a cat with chronic diarrhea.

Procedure—Optimal conditions for use of the culture system, such as quantity of fecal inoculum (0.025 to 0.2 g) and cultivation temperature (25 or 37°C [98.6 or 77.0°F]), were determined. Specificity of the system was examined by attempted culture of *Giardia lamblia* and *Pentatrichomonas hominis*. Sensitivity of the system to detect *T foetus* was determined by inoculation of culture system pouches with serially diluted *T foetus* suspensions with and without feces.

Results—Detection limit of the culture system was 1 and 1,000 *T foetus* organisms without and with feces from cats, respectively. Optimal fecal inoculum was < 0.1 g of feces. At 37°C, cultures yielded positive results in 24 hours; organisms remained viable for 1 to 6 days, and bacterial overgrowth was common. At 25°C, cultures yielded positive results in 1 to 11 days; organisms were long-lived, and bacterial overgrowth was uncommon. Neither *G lamblia* or *P hominis* survived in the culture system.

Conclusions and Clinical Relevance—The culture system was sensitive and specific for culture of *T foetus* in feces of cats. Performance was optimal when test kits were inoculated with ≤ 0.1 g of freshly voided feces and cultured at 25°C. (*J Am Vet Med Assoc* 2003;222:1376–1379)

Tritrichomonas foetus is a flagellated protozoan parasite that is an important venereal pathogen of naturally bred cattle. The organism is transmitted from the prepuce of the bull to the vagina and uterus during coitus, and the resultant infection leads to infertility and abortion.¹ Recently, *T foetus* has been identified as

an intestinal pathogen in domestic cats from which intraluminal infection of the colon leads to chronic large-bowel diarrhea.^{2,4} Infected cats are usually young and frequently reside in densely populated housing such as catteries or animal shelters.² Cats often have a history of infection with *Giardia* spp; these infections are subsequently identified as trichomonosis after failure to eradicate the organisms with standard antiprotozoal treatment (eg, metronidazole or fenbendazole).² At this time, there is no effective antimicrobial treatment for *T foetus* infection, and the origin of the infection in cats is unknown.

Diagnosis of trichomonosis in cats is made by observation of trichomonads in samples of freshly voided feces that are suspended in physiologic saline (0.9% NaCl) solution and examined microscopically at 200 to 400× magnification. *Tritrichomonas foetus* can also be grown from feces via incubation at 37°C in Diamond's medium, although this test is not commercially available as a kit and seems to be impractical for use in clinical settings. The sensitivity of direct examination of a fecal smear for diagnosis of *T foetus* in naturally infected cats is unknown but suspected to be poor. In a study³ of cats with chronic experimentally induced *T foetus* infection, trichomonads were observed in only 2% (4/192) of microscopic examinations of fecal smears. Diagnostic techniques that are presently available also lack sufficient specificity. Upon microscopic examination of fecal smears, *T foetus* are often mistaken for *Giardia* spp presumably because of unfamiliarity with the appearance of *T foetus*.² Although these organisms are similar in size and flagellated, careful microscopic examination reveals *Giardia* organisms to have motility that resembles the fall of a leaf, whereas trichomonads have erratic motion and a distinct undulating membrane that extends the full length of the organisms. Perhaps more importantly, *T foetus* cannot be reliably distinguished from nonpathogenic intestinal trichomonads such as *Pentatrichomonas hominis* on the basis of light microscopic appearance.^{2,5,6} *Pentatrichomonas hominis* can be found in the intestinal tract of several species; the organism is considered to be opportunistic, commensal, and highly susceptible to antimicrobial treatment.⁷ Thus, definitive identification of trichomonads in feces of cats with diarrhea is instrumental in assessment of the need for further diagnostic investigations, likely response to antimicrobial treatment, and prognosis.

A culture system^a for diagnosis of *T foetus* infection in cattle is commercially available.⁸ The culture system is supplied in individual test pouches; these pouches are made of clear plastic through which the contents

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can be viewed microscopically and contain a proprietary culture medium. To screen cattle for *T foetus* infection, pouches are inoculated under field conditions with samples of prepuccial smegma or vaginal mucus. After inoculation, the pouch is sealed and used for sample transport, selective cultivation, and diagnosis by observation of *T foetus* in the pouch contents by use of a light microscope.

The purpose of this study was to determine whether the commercially available culture system could be used for cultivation of *T foetus* from feces of cats and to determine optimal conditions for protozoal culture of feces via that system. In addition, the study was intended to assess the sensitivity and specificity of the culture system for detection of *T foetus* in feces and to compare the ability of the culture system to detect *T foetus* infection in fecal samples obtained from a large group of at-risk domestic cats with that of microscopic examination of fecal smears and protozoal culture of feces in modified Diamond's medium.

Materials and Methods

Protozoa—*Tritrichomonas foetus* was isolated by protozoal culture from the feces of a 2-year-old spayed female domestic longhair cat that had chronic large-bowel diarrhea. Briefly, a sample (0.1 g) of feces was suspended in 10 mL of sterile phosphate-buffered saline solution (PBSS; pH 7.3). An aliquot (100 μ L) of this solution was inoculated into a culture tube containing 10 mL of antimicrobial-fortified modified Diamond's medium^{9,b} (penicillin [10⁶ U/L], streptomycin [15 g/L], and amphotericin B [2 mg/L]) and incubated at 37°C (98.6°F). The isolate was identified as *T foetus* via analysis of a partial sequence of ITS1 and 5.8S rDNA that was amplified by polymerase chain reaction (PCR) assay using *T foetus*-specific primers, as described.¹⁰ *Pentatrichomonas hominis* (American Type Culture Collection [ATCC]^c 30098) and a feline isolate of *G lamblia* (ATCC 50163) were grown in recommended media at 37°C prior to inoculation into the culture system.

Culture system technique—Fecal samples were inoculated into the pouches according to manufacturer instructions and incubated in an upright position at either 37 or 25°C (77°F). Prior to microscopic examination, pouches were tapped against a bench-top to dislodge adherent organisms and clamped in a device provided by the manufacturer that allows the pouch to be mounted on the stage of a light microscope (Fig 1). Pouches were examined at 200 \times magnification with a light microscope; observation of motile *T foetus* organisms was considered a positive result.

Optimal fecal inoculum—Pouches were inoculated with 0.025, 0.05, 0.1, or 0.2 g (4 each) of freshly voided feces obtained from a domestic longhair cat with chronic diarrhea associated with *T foetus* infection. For each of the 4 inocula, 2 pouches were incubated at 37 and 25°C.

Optimal culture temperature and sensitivity—From the culture of *T foetus* (obtained from the clinically affected cat), organisms in the logarithmic phase of growth were washed 3 times in sterile PBSS by centrifugation (1,500 \times g for 5 minutes) and counted by means of a hemocytometer and light microscope. Trichomonads were serially diluted in PBSS to concentrations of 10,000, 1,000, 100, 10, 1, 0, 0.1, and 0.01 *T foetus*/200 μ L. For each dilution, 4 pouches were inoculated with 200 μ L of suspension. Each pouch was assigned to 1 of 4 conditions for incubation: maintained at 37 or 25°C with or without 0.05 g of cat feces. This exper-

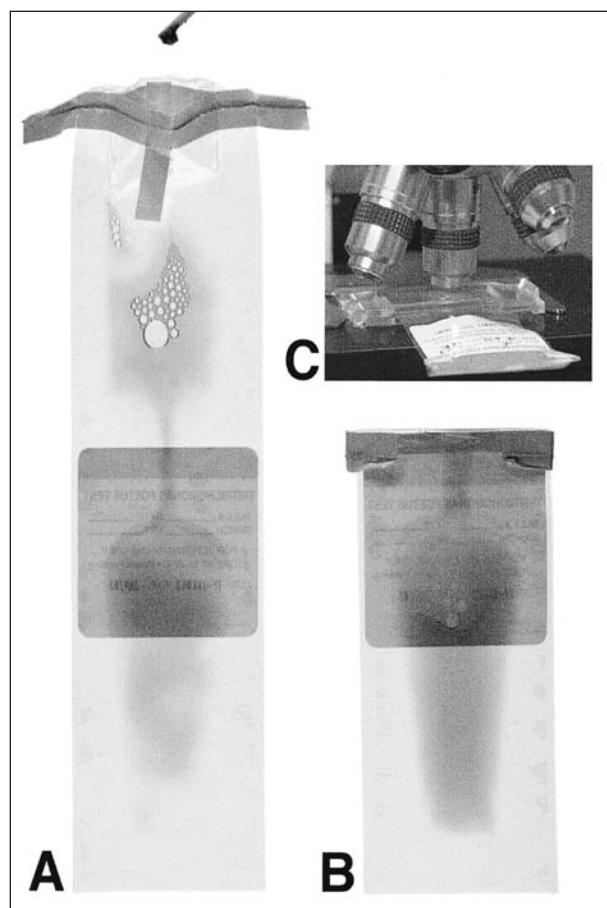


Figure 1—Photographs of a commercially available culture system used for detection of *Tritrichomonas foetus* in cat feces. A—The culture system is provided in a pouch that has 2 chambers containing culture media, connected by a channel. To inoculate the pouch, the upper chamber is opened and approximately 0.05 g of feces is mixed with the chamber contents. B—For cultivation, the top edge is rolled down to seal the opening and force the contents of the upper chamber into the lower chamber. C—Motile organisms can be viewed directly through the pouch at 200 or 400 \times magnification with a light microscope.

imental series was repeated so that 2 pouches for each dilution under each condition of culture were examined (64 pouches).

As part of an epidemiologic study^d of feline *T foetus* and *Giardia* spp infections, voided fecal samples were obtained from 117 purebred cats at an international cat show.^e For each sample, a fecal smear was prepared and examined immediately; a culture system pouch was inoculated with \leq 0.1 g of feces, incubated at 25°C, and examined microscopically at 200 \times for motile *T foetus* organisms daily. Additionally, 0.1 g of each fecal sample was suspended in 10 mL of sterile PBSS and transported overnight to our laboratory for protozoal culture in modified Diamond's medium, as described.^{2,3} Cultures yielding positive results were identified on the basis of results of PCR with *T foetus*-specific primers, as described.¹⁰

Specificity—Culture system pouches were inoculated with 200 μ L ($>$ 10,000 organisms) of log-phase *G lamblia* or *P hominis* and incubated at 37 and 25°C (3 replicates/organism per temperature).

Effect of fecal storage conditions on recovery of *T foetus*—A freshly voided fecal sample was obtained from

a cat with chronic large-bowel diarrhea attributable to *T foetus* infection. Two-gram portions of the fecal sample were placed in 3 sealed plastic containers (fecal specimen cups) with pelleted recycled corn cob litter,[†] 3 mL of sterile physiologic saline (0.9% NaCl) solution, or no additive. Daily, feces from each container were suspended in physiologic saline solution and examined with a light microscope at 200× magnification; if microscopic examination revealed no trichomonads, 0.05 g of the feces was inoculated into a culture system pouch as described. These examinations were continued until culture system pouches yielded negative results on 3 consecutive days.

Results

In the absence of cat feces, the culture system yielded positive results after inoculation with only 1 *T foetus* organism. Supplemented with 0.05 g of feces, the culture system yielded positive results after inoculation with $\geq 1,000$ *T foetus* organisms. The optimal quantity of fecal inoculum was 0.025 to 0.05 g of feces (a volume that is approx equivalent to that of a peppercorn). Pouches inoculated with > 0.1 g of feces (a volume that is approx equivalent to that of a pea) had overgrowth of gas-producing bacteria or yeast within 24 hours, regardless of incubation temperature. Neither *G lamblia* or *P hominis* organisms survived in the culture system for > 24 hours at either 25 or 37°C.

When incubated in pouches at 25°C, feces (≤ 0.1 g/pouch) from 21 naturally infected cats yielded positive results in 1 to 11 days (median, 3 days) and were found to contain viable *T foetus* organisms for up to 4 months. In the culture system pouches, overgrowth of fecal bacteria was uncommon (observed in 12/117 [10%] cultures), and onset of bacterial overgrowth was delayed (median time to bacterial overgrowth, 8 days; range, 2 to 12 days). When incubated in pouches at 37°C, feces (≤ 0.1 g/pouch) containing *T foetus* yielded positive results within 24 hours and were found to contain viable *T foetus* organisms for only 1 to 6 days (median, 2 days; $n = 14$ samples). At 37°C, overgrowth of fecal bacteria in culture system pouches was common (observed in 7/14 cultures) and developed rapidly (median time to bacterial overgrowth, ≤ 3 days; range, 1 to 3 days). On the basis of analysis of partial ITS1 and 5.8S rDNA gene sequences, trichomonads from culture system pouches that yielded positive results with feces obtained from naturally infected cats ($n = 19$) matched identically with *T foetus* (GenBank accession No. AF466751).

Compared with immediate culture in pouches, next-day culture of PBSS-diluted feces shipped overnight in modified Diamond's medium was less sensitive for detection of *T foetus* infection; of 117 fecal samples, 20 yielded positive results in culture system pouches, whereas 9 yielded positive results after culture in modified Diamond's medium. Examination of fecal smears was least sensitive for detection of *T foetus* (5/117 fecal samples yielded positive results). The number of days that *T foetus* was recovered from fecal samples stored at 25°C in sealed plastic containers with pelleted litter, no additive, or saline solution was 0, 1, and 2 days, respectively, as determined by means of fecal smear examination and 0, 1, and 4 days, respectively, as determined with the culture system.

Discussion

In the diagnosis of *T foetus* infection in cats, protozoal culture of feces with the culture system has several distinct and practical advantages, compared with use of modified Diamond's medium. These advantages include commercial availability of the pouch system, no requirement for dilution or shipment of feces (processes that are anticipated to diminish diagnostic sensitivity), and no requirement for preparation of sterile media or facilities for incubation of cultures at 37°C. The ability to view the contents of the sealed pouch directly with a light microscope reduces exposure of personnel to large numbers of *T foetus* organisms. By use of aliquots of serially diluted suspensions of cultured *T foetus* without or with cat feces, the detection limit of the culture system was determined to be 1 and $\geq 1,000$ *T foetus* organisms, respectively. The culture system's detection limit in the absence of feces is comparable to that reported by Borchardt et al,⁸ who determined an absolute sensitivity of ≤ 100 organisms by use of cultured *T foetus* isolates from cattle. Inclusion of cat feces reduced the sensitivity of the culture system for detection of *T foetus*. This result was expected, because feces contain large numbers of competitive microorganisms. In culture system pouches that contained ≤ 0.1 g of cat feces and were incubated at 37°C, overgrowth with fecal bacteria or yeast developed rapidly, and the cultures were not useful diagnostically. However, in culture system pouches that were inoculated with ≤ 0.1 g of feces and cultured at 25°C, growth of competing fecal microflora was notably inhibited. The practical importance of the culture system's detection limit of $\geq 1,000$ organisms/0.05 g of feces at an incubation temperature of 25°C is difficult to determine, because the mean number of organisms shed by cats infected with *T foetus* is unknown. Nevertheless, our experience has suggested that the number of *T foetus* organisms shed by cats with clinical signs of disease is great enough to be routinely detected by the culture system. In bulls known to be infected with *T foetus*, the sensitivity of the culture system for detection of *T foetus* was 88%.¹¹

Among the 117 show cats included in our study, the culture system detected more cats with *T foetus* infection ($n = 20$) than did protozoal culture of feces in modified Diamond's medium (9). Importantly, the differences in practical application of these 2 culture methods may have influenced this finding. Culture system pouches were inoculated directly with ≤ 0.1 g of feces and cultured at 25°C, whereas prior to culture in Diamond's medium at 37°C, feces were first diluted 100-fold (to prevent bacterial overgrowth) and transported overnight to a laboratory. Thus, these results do not allow a direct comparison of absolute sensitivities for detection of *T foetus* of the 2 culture methods. In other studies,^{8,12-14} the diagnostic sensitivity of the culture system equaled or exceeded that of Diamond's medium for detection of isolated *T foetus* organisms.

In our study, the culture system did not support growth of either *P hominis* or an isolate of *G lamblia* obtained from a cat. This observation is clinically important, because *T foetus* is difficult to distinguish from *P hominis* and *Giardia* spp and is much less amenable to

treatment than those organisms. In the study reported here, all culture system isolates were identified as *T foetus* by PCR assay. On the basis of these findings, it is reasonable to conclude that fecal samples obtained from cats with diarrhea that yield positive results in the culture system are unlikely to contain *P hominis* or *Giardia* spp and are likely to contain *T foetus*. However, the types of trichomonads for which cats are hosts and the specificity of the culture system with regard to detection of these other types of trichomonads are unknown. Finally, growth of *T foetus* in the culture system does not preclude the possibility of intestinal coinfection with *P hominis* or *Giardia* spp.

Our data regarding optimal storage of feces for later recovery of *T foetus* indicated that desiccation had a negative effect on *T foetus* viability. Feces placed in contact with litter^d desiccated rapidly, and *T foetus* organisms were not viable after 24 hours. In the absence of litter, *T foetus* remained viable in fecal samples for 24 hours; in physiologic saline solution, *T foetus* remained viable in fecal samples for 4 days. This difference appeared to be directly attributable to maintenance of fecal moisture content. In our study, investigation of optimal fecal storage conditions was conducted at 25°C, because it is known that *T foetus* does not survive conditions of refrigeration (4°C [39.2°F]) or freezing (−20°C [−4°F]).¹⁴

On the basis of our findings, we recommend in-clinic use of the culture system in combination with microscopic examination of fecal smears for diagnosis of *T foetus* infection in cats. Cats that should be tested for *T foetus* infection include young cats from dense housing conditions that have clinical signs of chronic large-bowel diarrhea. Cats with *Giardia* spp detected by use of fecal smear examination that fail to respond to antimicrobial treatment should be reassessed for *T foetus* infection, because the observed trophozoites may have been incorrectly identified initially. To detect *T foetus* in fecal samples, culture system pouches should be inoculated with approximately 0.05 g of freshly voided or loop-collected feces and incubated at 25°C in an upright position. Pouch contents should be examined for motile *T foetus* at 200 to 400× magnification with a light microscope. We recommend examination of pouch contents every 48 hours; pouches can be discarded if they continue to yield negative results after 12 days. If the fecal sample has to be transported or stored prior to culture, viability of *T foetus* can be extended by the addition of physiologic saline solution (approx 3 mL of saline solu-

tion/2 g of feces). Fecal samples should also be free of cat litter, because this may desiccate the sample and kill the organisms.

^aInPouch TF, Biomed Diagnostics Inc, San Jose, Calif.

^bModified Diamond's medium, Remel, Lenexa, Kan.

^cAmerican Type Culture Collection, Rockville, Md.

^dGookin JL, Stebbins ME, Adams E, et al. Prevalence and risk of *T foetus* infection in cattery cats (abstr). 21st Annu Forum of the Am Coll Vet Intern Med, Charlotte, NC, June, 2003.

^eCat Fanciers Association international cat show, Houston, Tex, November, 2001.

^fBed-O-Cobs, The Andersons, Maumee, Ohio.

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