

This article or any portion thereof cannot be reproduced for other than individual use nor posted to any other website without written permission of the authors.

# AN OWNERS GUIDE TO DIAGNOSIS AND TREATMENT OF CATS INFECTED WITH *TRITRICHOMONAS FOETUS*

Jody L. Gookin, DVM, Ph.D., DACVIM (Internal Medicine)  
North Carolina State University  
College of Veterinary Medicine  
[http://www.cvm.ncsu.edu/docs/jody\\_gookin.html](http://www.cvm.ncsu.edu/docs/jody_gookin.html)

Dave Dybas  
HighgaitsPaws Abyssinians  
Clintondale, New York  
<http://www.highgait.com>

*Tritrichomonas foetus* is an emerging parasite of felines. This guide is a merger of experimental findings, clinical observations, anecdote, and breeder experience from which the authors have attempted to answer the most common questions and to provide practical recommendations to cat owners. Currently, no assurances can be given that following the recommendations in this guide will result in flawless determination of which cats are infected with *T. foetus* nor will it guarantee that *T. foetus* can be eradicated from any given cat or cattery.

Continue to check this webpage ([http://www.cvm.ncsu.edu/docs/jody\\_gookin.html](http://www.cvm.ncsu.edu/docs/jody_gookin.html)) for updated versions of this guide. This is version #4 (updated April 28, 2008).

<u>INDEX</u>	<u>PAGE NUMBER</u>
Background on <i>T. foetus</i> infection	1
Diagnosis of <i>T. foetus</i> infection	3
Treatment of <i>T. foetus</i> infection	6
Management of <i>T. foetus</i> infection	9
Do-it-yourself testing for <i>T. foetus</i> infection	11
Publications on feline <i>T. foetus</i> infection	15

## BACKGROUND ON *T. FOETUS* INFECTION

### What is *T. foetus*?

*Tritrichomonas foetus* is a recently recognized parasite in the feline world. It is a single-celled protozoan that likes the warm, moist, and oxygen-deprived conditions inside the feline colon. Testing for this parasite is still not routine at most veterinary clinics. *T. foetus* looks similar to *Giardia* when viewed under a microscope. Therefore, its misdiagnosis as *Giardia* is common. Fecal floats and *Giardia* SNAP tests do not detect *T. foetus*; a *T. foetus*-specific test must be performed to detect it.

### Where did *T. foetus* come from?

*Tritrichomonas foetus* is well known as a venereal infection of bovines (cattle). It was first reported in cats in 1996, where it was found in the intestine and was associated with diarrhea. There is no evidence that feline *T. foetus* came from cattle, in fact, veterinarians don't know how

*T. foetus* made its way into the feline population.

**How common is *T. foetus* infection?**

*T. foetus* is common in purebred and shelter cats, with no particular breed being over represented. No breed of cat is known to be immune to *T. foetus*. Based on a survey of cats at an international cat show, approximately a third of the purebred feline population may be infected with *T. foetus*. Geographically, *T. foetus* has been found in many countries.

**What are the symptoms of *T. foetus* infection?**

Infected cats may or may not have observable symptoms. In cats showing symptoms, *T. foetus* causes diarrhea, characterized by cow pie-like stools that are often gassy and malodorous. Sometimes there can be mucus or fresh blood in the stool or feces can dribble from the anus. Several breeders have commented that in symptomatic cats the stool has a very strong and unpleasant odor. Importantly, absence of diarrhea does not mean that a cat is free of infection, particularly in multi-cat households where another cat may have tested positive *T. foetus*. Adult cats appear to be less inclined to develop diarrhea in response to *T. foetus*, but nevertheless may serve as a source of the infection for others.

**How do cats get *T. foetus*?** The primary infection pathway is probably the litter box where a well-timed use by two cats can transfer the parasite from the feces of one cat to the paws of another where they later become ingested during grooming. *T. foetus* can live for several days in a wet stool (wet is the key word). Mutual grooming may also transfer the parasite.

**How will *T. foetus* affect the health of my cat?** Infected cats usually do not have their overall health adversely affected. Therefore, owners of infected cats often ignore the infection since their cats maintain body and coat condition. Unfortunately, such cats remain a source of infection for others. If left untreated, approximately ninety percent of infected cats will stop having diarrhea within two years. However, most of these cats will continue to carry the *T. foetus* organism, possibly for a lifetime. Single cat owners may find this outcome satisfactory if the cat remains healthy in other respects. However, if left untreated the *T. foetus* infection will perpetuate within their cattery and spread to other cats and catteries when kittens are adopted or animals are transported for breeding. Although many cats will eventually resolve their diarrhea, the prolonged presence of the organisms could predispose to development of inflammatory bowel disease later in life, but this has not yet been explored.

**Can *T. foetus* infect people?** In light of the intimate association between infected cats and their human companions, the potential for zoonotic transmission should be considered. Only a single case of human infection with *T. foetus* appears in the literature. The infection was present in the central nervous system of a man who was immunosuppressed and had undergone a peripheral blood stem cell transplantation.

## DIAGNOSIS OF *T. FOETUS* INFECTION

### Who should test for *T. foetus*?

Catteries that currently or periodically have cats with bouts of diarrhea and that have passed tests for other parasites such as *Giardia*, Helminths (worms), and Coccidia. Catteries should also consider baseline testing their cats even if there has been no instances of diarrhea lately. All new cats coming into the cattery should also be tested for *T. foetus*.

### How can I tell if my cat has *T. foetus* infection?

There are 3 ways to test for *T. foetus* infection.

1. **Direct Fecal Smear:** Fresh feces are examined in saline under a microscope for the presence of trichomonads.

Cost – cheapest

Sensitivity – poor

Possible reasons for failure to detect infection –

- Use of old, dried out, refrigerated or litter-contaminated feces
- Specimen contains only few *T. foetus* organisms that escape detection
- *T. foetus* misidentified as *Giardia*
- Cat is currently or has recently (within past 7-days) received antibiotics

Skill level – Examiner must be capable of differentiating between similar looking organisms such as *Giardia*. Should be performed by a veterinarian or diagnostic laboratory. A video of *T. foetus* and *Giardia* as observed under the microscope can be viewed at

[http://www.cvm.ncsu.edu/docs/jody\\_gookin.html](http://www.cvm.ncsu.edu/docs/jody_gookin.html)

2. **Fecal Culture:** Voided feces and/or a specimen obtained by a rectal swab or fecal loop is incubated in a growth medium. Included in this medium are antibiotics, which suppress unwanted bacterial growth. The culture is then examined microscopically for the presence of *T. foetus*. The only commercially available culture test kit is BioMed's Feline InPouchTF™ test kit.

Cost – low; \$5-\$7 per culture pouch depending on quantity purchased. Veterinary charges for sample collection, incubation, and examination will vary.

Sensitivity – very good; if *T. foetus* organisms are present their numbers will multiply in the culture over time, increasing the likelihood of their detection.

Possible reasons for failure to detect infection –

- Use of old, dried out, non-diarrheic, litter-contaminated or refrigerated feces in which *T. foetus* are unlikely to have survived
- Use of fecal loop lubricants containing antiseptics
- Cat is currently or has recently (within past 7-days) received antibiotics
- Pouch was not incubated at 37°C for at least 24 hours or was subjected to conditions that caused the parasites to die

Skill level – Ability to use a microscope. Since the InPouchTF test is selective, i.e. it will not support similar appearing organisms such as *Giardia* or *P. hominis*, the examiner need not have expertise in organism identification.

Comment – Currently the most user friendly testing method to detect *T. foetus*. Do-It-Yourself testing will lower testing cost considerably. However, since live organisms are required to inoculate the pouch and optimum growth conditions for the organisms must be maintained during the test period, the user needs to use care in handling both the specimen and the pouch or the test may produce a false negative result (see above).

For information on purchase of pouches go to [www.biomeddiagnostics.com](http://www.biomeddiagnostics.com). This test can be purchased without a prescription.

3. **Polymerase chain reaction (PCR):** This is a molecular biology test that is used to identify trace amounts of *T. foetus* DNA in the feces.

Cost – expensive; \$75 per test depending on the laboratory. This does not include shipping or potential mark-up in price by your veterinarian.

Sensitivity – excellent; can detect both live and dead organisms.

Possible reasons for failure to detect infection –

- Use of old, dried out, non-diarrheic, or litter-contaminated feces
- Cat is currently or has recently (within past 7-days) received antibiotics
- Insufficient quantity of sample submitted

Skill level – expert.

Comments – currently the most sensitive test in detecting infected cats. The sample must be submitted by your veterinarian. Detailed information on sample submission requirements for PCR testing can be found at [http://www.cvm.ncsu.edu/docs/jody\\_gookin.html](http://www.cvm.ncsu.edu/docs/jody_gookin.html)

**Important Disclaimer** – No diagnostic tests are available that will detect infection 100% of the time. If the test results are positive, the cat has *T. foetus* infection. If the test results are negative, the cat likely does not, but still could have *T. foetus* infection. Importantly, negative test results cannot be used to eliminate the possibility of this infection.

**How can I maximize the chance of finding *T. foetus* infection in my cat?**

The best chance of finding *T. foetus* is **1)** to use the most sensitive testing method = PCR, **2)** consider testing repeatedly if your suspicion is high, **3)** only submit fecal samples that are

obtained when cats are having clinical signs of diarrhea, **4)** make sure the cat has not received any antibiotics for at least 7 days prior to fecal collection, **5)** have your veterinarian obtain the fecal sample directly from the colon using a fecal “loop” or saline flush of the colon.

### **How should I obtain the fecal specimen?**

Fecal samples should always be fresh, free of contaminating litter, and kept unrefrigerated prior to testing. If a stool sample is being transported to a veterinary clinic, keep it warm and moist. *T. foetus* organisms perish quickly at temperatures below 60°F or above 105°F. There are four options for obtaining the fecal sample.

**Litter box specimen:** Use clean, non-clumping litter and isolate cat (e.g. cage) to ensure that the specimen will not be misidentified and/or contaminated by another cat. Fresh, witnessed stools are preferable: avoid using stools that are hours old. The wetter the stool the longer the *T. foetus* will survive outside of the cat's body. Always use the wettest part of the stool from which to extract a specimen. If necessary, sample the interior of the stool if the outside is dry or contaminated. Specimens obtained from dry or semi-dry stools should be avoided, in which case a loop or rectal swab method to gather a specimen should be substituted.

**Fecal loop:** Using a fecal loop or colonic flush is the preferred method for collecting a specimen for testing. Unless skilled at using a fecal loop, it is recommended that a kitten/puppy size loop be used to perform a shallow loop of the cat's rectum. Feces may not be readily available when a smaller loop is used but the loop may gather mucus from the lining of the colon. This is usually sufficient to inoculate a culture pouch. Do not use lubricants containing antiseptics, e.g. K-Y jelly. Use either sterile saline, the liquid media from the pouch or Aplicare Jelly ([http://www.aplicare.com/catalog/index.php?cPath=1\\_103\\_10304](http://www.aplicare.com/catalog/index.php?cPath=1_103_10304)).

**Saline flush:** To perform a saline flush, a red-rubber catheter is inserted per rectum into the proximal (way up there) colon. Approximately 10 cc of sterile saline is injected through the catheter into the colon and then gently re-aspirated. The solution can then be examined directly under the microscope for trichomonads or a drop can be placed in a fecal culture pouch. Alternatively, the contents can be sedimented in a centrifuge and submitted for PCR analysis.

**Rectal swab:** Wet a sterile Q-tip with sterile saline and insert it into the rectum. Fecal material need not be obtained; the lining of the colon is a rich source of *T. foetus* so the mucus gathered by the swab will be sufficient to inoculate a pouch, although insufficient if being submitted for PCR.

**\*\*\*Note – *Serious injury or perforation of the colon can result from improper use of a fecal loop and/or swab. It is highly recommended that you seek training from your veterinarian if planning on performing these procedures on your own.***

### **Can I test my own cats for *T. foetus*?**

Yes. For detailed guidelines for do-it-yourself testing, please see **Appendix A** at the end of this manuscript.

## TREATMENT OF *T. FOETUS* INFECTION

**Disclaimer** – Treatment of *T. foetus* infection should never be undertaken except in cases of confirmed infection and under the informed advisement of your personal veterinarian.

### **Is there any treatment for feline *T. foetus* infection?**

Yes. Ronidazole has been shown to be an effective treatment when administered to cats that were experimentally infected with *T. foetus*.

### **What is the dose for Ronidazole?**

Recent data gathered on the pharmacokinetics of Ronidazole in cats suggest that 30-mg/kg orally once a day for 14 days is a reasonable regimen. It is important that the amount of Ronidazole be accurately calculated for each cat on the basis of its body weight. There is no evidence that higher doses of Ronidazole or doses given for a longer period of time are more effective. In fact, higher doses will greatly increase the likelihood of a neurological reaction to the drug.

### **Is Ronidazole safe?**

Toxicity data have not been published for this drug. However, neurotoxicity has resulted in some cats treated with Ronidazole. Signs of toxicity include lethargy, inappetence, ataxia (drunken-like behavior), or seizures. These generally resolve if the drug is withdrawn immediately, but can last 1-2 weeks and may require costly and intensive emergency veterinary care. Cats need to be monitored closely while receiving Ronidazole. Signs of neurotoxicity may be easier to spot if the treated cat is engaged each day in a playful activity (e.g. laser pointer) that requires coordination and agility. If symptoms of toxicity are observed, owners must resist the temptation to continue/complete treatment with Ronidazole. Even after Ronidazole is stopped the symptoms of neurotoxicity may continue to worsen for the next few days before slowly subsiding. Continuing treatment after signs of toxicity are observed will be very dangerous and life threatening to your cat.

Ronidazole is not FDA approved for use in companion animals. It is currently banned for use in food-producing animals in countries outside of the United States due to human hazards. Due diligence is required for protection of humans from exposure to Ronidazole and veterinarians are advised to obtain informed consent prior to use of this drug in cats.

### **What should I do if my cat shows signs of toxicity while receiving Ronidazole?**

Stop the drug immediately and contact your veterinarian. Depending on the severity of signs your veterinarian may advise that your cat can be managed at home or alternatively, need hospitalization for more intensive care. These decisions are made on a case-by-case basis based on your veterinarians best judgment and therefore cannot be further elaborated upon here.

### **Where can I get Ronidazole?**

Veterinarians can write a prescription for Ronidazole to be compounded by various pharmacies around the country. A prescription is required. Ronidazole can also be obtained as a 10% active-drug powder used to treat trichomoniasis in pigeons. This tastes terrible and is hard to administer to cats in sufficient quantities to be effective. We do not recommend it.

### **Ronidazole Suppliers:**

#### United States:

Numerous compounding pharmacies will fill a prescription for ronidazole. For a list of veterinary compounding pharmacies and their contact information, see the following website: [http://www.marvistavet.com/html/compounding\\_pharmacies.html](http://www.marvistavet.com/html/compounding_pharmacies.html). Note - this list is not inclusive of all pharmacies and has not been pre-screened to include only those pharmacies from which ronidazole can be obtained.

#### Canada:

Haber's Compounding Pharmacy, Toronto, Ontario (416-656-9800)

McDonald's Pharmacy, Vancouver, Canada (604-734-4311)

### **Formulations available:**

**Capsules:** Preferred, since it is the most accurate method of administering Ronidazole. Most pharmacies custom fill the capsules to the dose prescribed by your vet. Some pharmacies are now offering an alternative, at a bit lower cost, by offering a range of pre-filled capsules. Your veterinarian selects the amount closest to what he/she wants prescribed. If several cats are to be treated at one time, do not use a one-dose-for-all approach wherein some cats will be under- and others over-dosed. This will predispose to treatment failures and toxicities.

**Liquid:** Ronidazole is very bitter so it is difficult to mask its taste. However for those un-pillable cats out there, a liquid formulation may be the only alternative. The problem with liquid is that the cat is expected to accept 14 doses of Ronidazole. If a portion of the dose is spit out, it is very difficult to determine how much was lost. This leaves open the possibility of under dosing or worse, overcompensating for the amount thought lost and therefore increasing the risk of neurotoxicity.

### **Can I give Ronidazole to nursing queens or very young kittens (<12-weeks)?**

Not recommended. Ronidazole is believed to be passed in the milk and young kittens may be at higher risk for neurotoxicity.

### **Can I give Ronidazole to pregnant queens?**

Not recommended. There is a possibility that Ronidazole may lead to birth defects or neurotoxicity in the unborn kittens.

### **Do I need to do any special disinfection of the cattery?**

*T. foetus* is a fragile organism whose life span outside the body is normally less than an hour although it can live for several days in moist stools. This lack of hardiness is due to the fact that *T. foetus* cannot form a cyst (as can *Giardia*) and does not like the presence of oxygen. If *T. foetus* dries out, if it is refrigerated or if it experiences temperatures above 105°F it will die. Keeping surfaces clean and dry is most important. During treatment, litter should be replaced and boxes disinfected on several occasions to prevent cats from re-infecting themselves with *T. foetus* shed during the treatment period.

### **How soon after treatment with Ronidazole can I expect the diarrhea to resolve?**

It is typical for cats with *T. foetus* infection to show some improvement in the diarrhea during the course of treatment. If diarrhea persists for greater than 14-days after treatment, the cat should be re-tested for *T. foetus*. If test results are negative for *T. foetus*, consider other causes of diarrhea including concurrent infection, dietary intolerances etc. *T. foetus* can cause considerable inflammation in the colon and it often takes several weeks for this to resolve after the organisms are killed.

### **I treated my cat with Ronidazole and it still tests positive for *T. foetus* – what do I do now?**

There are several possibilities should be considered for why *T. foetus* infection may persist after treatment.

- Ronidazole was given at too low of a dose (< 30 mg/kg once a day for 14-days), the cat did not ingest a sufficient amount of the drug (e.g. spat out liquid), or an inappropriate formulation of the drug (e.g. 10% powder formulation for pigeons) was prescribed.
  - Consider repeating treatment with an appropriate dose/formulation of Ronidazole.
- The cat has been reinfected with *T. foetus* by another cat in the household that may or may not be showing any signs of infection. A common misconception is that only cats with diarrhea are infected.
  - The cat should be isolated from all other cats during and after treatment. Other cats should be tested for *T. foetus*.
- The cat is infected with *T. foetus* that are “resistant” to Ronidazole. How often an appropriate treatment course with Ronidazole actually fails to kill *T. foetus* is unknown.
  - Completely rule out the above causes and consider repeating a course of Ronidazole before assuming your cat is resistant to treatment.

### **My cat got sick while receiving Ronidazole and the drug had to be stopped prematurely.**

**Should I try the treatment again?** No. It is possible that the Ronidazole given was still enough to kill the *T. foetus*. Re-test the cat and find out if the infection is still there. If the infection persists, and the dose given was appropriate (30 mg/kg PO once a day), it is likely that toxicity will recur if you try to repeat the treatment. If the treatment was at an inappropriately high dose ( $\geq 50$  mg/kg PO once a day) you can consider repeating the treatment at the correct dose – but must do so with extreme caution.

### **Is Tinidazole effective against *T. foetus* infection?**

No. Tinidazole was ineffective at eradicating *T. foetus* from cats that were experimentally infected with the organism and has not worked well at clearing the infection from naturally infected cats.

### **If I cannot cure my cat of *T. foetus* infection or choose not to treat, is there anything I can do to control the diarrhea?**

Unfortunately, many different approaches have been tried without success to control the diarrhea including changes in diet, use of different antibiotics, and supplementation with nutraceuticals and probiotics. However, there have been no controlled studies of any of these therapies. It has actually been suggested that frequent changes in diet and indiscriminant use of antibiotics actually prolongs the time it takes for cats to eventually resolve the diarrhea on their own.

### **If I don't choose to treat my cat's *T. foetus* infection can my cat self-cure?**

Unlikely, although the diarrhea may resolve with time, the infected cat will probably remain a carrier and a source of infection for other cats. How much of a threat they will be to other cats is not known. Some cats who remain asymptomatic carriers seem to be low shedders of the *T. foetus* organism.

## **APPROACH TO MANAGEMENT OF *T. FOETUS* INFECTION IN THE CATTERY**

### **How can I identify *T. foetus* infected cats from uninfected cats in my cattery?**

Repeated testing, performing tests appropriately, and appropriate efforts to isolate infected cats to avoid transmission of the infection or re-infection are essential to efforts to identify all of the infected cats in the cattery.

Here is one possible approach:

- Make sure that all cats to be tested have not received any antibiotics such as Flagyl (metronidazole) for the past 14 days. These types of drugs may temporarily reduce the number of *T. foetus* and cause a false negative test result.
- Isolate cats in which *T. foetus* infection may be suspected, such as those with clinical signs of diarrhea.
- If your facility allows, you may want to divide the initial test group in smaller sub-groups. This will minimize sampling errors and limit the number of possible re-infections if a treatment fails. Cats in any group should be compatible and of the same gender.
- Wherever possible, use the preferred method, a fecal loop, to obtain a test specimen from each cat, in every group.

### **How certain can I be of the *T. foetus* test results?**

A positive test result indicates that a cat has *T. foetus* infection. A negative test result can never *prove* that your cat is not infected as no diagnostic test is capable of detecting the infection 100% of the time. Negative test results obtained on multiple occasions builds the strongest case for the absence of *T. foetus* infection in a given cat.

Here are some considerations that may help you evaluate the test results:

- If all cats are tested, no cats test positive, and signs of diarrhea are not present in the cattery, then *T. foetus* infection is unlikely.
- If all cats are tested, no cats test positive, and signs of diarrhea are present in the cattery, retest the cats with diarrhea. Evaluate and recheck the methods used to collect the specimen and perform the test. Consider using a more direct (e.g. fecal loop or rectal swab) and sensitive (PCR) method to obtain the specimen for a second test.
- Be sure to take into account the most recent exposures and the incubation period of *T. foetus* (assume ~21 days) when your test results are mixed (i.e. positives and negatives).

**Note:** If any cats test positive, they should be isolated from the rest of the group. Any cats they were previously in contact with the infected cat are at high risk for also being infected. These other cats should be tested repeatedly for *T. foetus* infection.

### **How do I get *T. foetus* out of my cattery?**

Reinfection with *T. foetus* is a common problem in catteries. Many cats are infected and do not show clinical signs. Thus, treating only the cats with diarrhea or confirmed infection will generally be ineffective unless the treated cats are isolated or removed from the cattery. Once an infection is discovered all cats in the cattery must be tested. Treating all cats in a cattery with Ronidazole without a confirming test is problematic as pregnant and nursing queens and very young kittens should not be treated with the drug, the likelihood of toxicity will increase with the larger number of cats that are treated, there is no guarantee that treatment will be effective in all of the cats, and doing so may be cost-prohibitive.

### **What are the costs of treating *T. foetus*?**

Cost considerations are always a factor in multi-cat households. The financial impact of testing and possibly treating many cats are obvious. This may create a temptation to work independently of one's veterinarian. This would be ill-advised. For those interested, information on do-it-yourself testing can be found at the end of this document. Cost of the drug to treat a *T. foetus* infection has dropped markedly in recent months. It would be prudent to price shop the cost at several different compounding pharmacies as pharmacies with a new stock of the drug tend to charge the lowest prices.

### **Once I complete the drug treatment for *T. foetus*, what should I do next?**

Do not assume all cats are cured. Keep the treated cats isolated. Wait at least 14 days before re-testing to allow any persistent effects of the drug to subside. If all test negative, wait another 14 days and perform a second group-wide post treatment test. Periodic testing for this group is recommended. Relapse of infection may happen as long as 20+ weeks after treatment. To avoid reinfection it is advisable to not re-introduce treated cats to the general cattery population.

### **What should I do if a cat that has been treated breaks with diarrhea?**

First, realize that diarrhea in cats is not uncommon but a *T. foetus* infection must be considered as a possible cause. Isolating and testing any cats with diarrhea is highly recommended.

### **How can I tell if my cat has been cured of *T. foetus* infection?**

It is very difficult to prove that a cat has been cured of infection. We recommend that cats be repeatedly tested for *T. foetus* by either culture of feces (most practical) or PCR for follow-up periods of 20 or more weeks after completion of treatment.

### **What if I have a *T. foetus* infected pregnant female?**

Females cannot be treated while carrying or nursing kittens.

### **What can be done to prevent the kittens of a *T. foetus* infected female from becoming infected?**

Removing the kittens from the mother at birth is one obvious, but not recommended method. The risk of harm to kittens and mother would probably outweigh the benefits. Another approach, which has worked on several occasions, is to place a platform (i.e. roof) over the kitten's litter pan such that the mother is excluded but the kittens have free access. Place the mother's litter pan out of reach of the kittens. Longhair mothers who are symptomatic will require closer monitoring than shorthairs as they may bring fresh feces on their coats back to the kittens. A 'fanny wash' should be performed to prevent this carry-back.

When the kittens are weaned and it is safe to remove the mother, begin treatment of the mother. Begin testing of the kittens if symptoms are observed otherwise wait 14 days after separating the mother to allow for incubation of a possible infection.

## **APPENDIX A: GUIDELINES FOR DO-IT-YOURSELF TESTING FOR *T. FOETUS* INFECTION**

Dave Dybas  
HighgaitsPaws Abyssinians  
Clintondale, New York  
<http://www.highgait.com>

**Disclaimer** – Provision of the following information should not be interpreted as recommending or condoning do-it-yourself testing. Do-it-yourself testing is highly prone to false-negative results. It cannot be used to prove that cats are not infected. Also, it is not unreasonable that other cattery owners, customers purchasing kittens, or veterinarians will be wary of the accuracy of the results of such testing. More credence should be given to finding positive results than to finding negatives.

For do-it-yourself testing, **BioMed Feline InPouchTF™** culture is the most practical. This test makes it feasible to do your own testing and you may gain a large cost savings when compared to diagnostic laboratory-performed tests.

No special skills are needed to detect *T. foetus* in a positive pouch culture. However, positive test results depend on finding live *T. foetus* in the feces, so appropriate care must be taken in obtaining a fresh specimen and maintaining the culture properly. *T. foetus* may be a stubborn adversary inside the body of a feline but it is very fragile outside of these confines so one has to keep *T. foetus* living to prevent the test results from becoming falsely negative.

**Precautions: All specimens should be handled in accordance with CDC-NIH recommendations for potentially infectious organisms, BIOHAZARD LEVEL 2. Refer to BioMed Diagnostics instruction brochure, which is shipped with the InPouchTF tests. Additional information on BIOHAZARD LEVEL 2 recommendations can be found at these websites:**

<http://bml.od.nih.gov/sect3bsl2.htm> (NIH)

<http://www.cdc.gov/od/ohs/biosfty/bml4/bml4s3.htm> (CDC)

### **How do I perform an InPouch test for *T. foetus*?**

#### **Gather the required equipment**

**Microscope:** A student microscope will suffice. Normally on this style of microscope three magnification objectives are provided (4X, 10X and 40X), which when combined with a 10X eyepiece lens provides total magnification levels of 40, 100 or 400X.

*T. foetus* is best found with 100X magnification. In addition, the microscope chosen must have adjustable illumination (e.g. iris or multi-holed disk). Student microscopes, can be

found on Ebay (either new or used) or from other web suppliers (<http://www.microscope-depot.com/seriesM.asp>). Cost: approx. \$100.

**Digital thermometer:** Since *T. foetus* is temperature sensitive, a thermometer that can display current temperature and record minimum and maximum temperature is a must. Indoor/Outdoor style thermometers work the best as these come with a detached temperature sensing element which can easily be placed alongside the pouches. The min/max memory feature will alert the user to temperature excursions that may have been lethal to the *T. foetus* organisms in the pouch and which could have biased the test results falsely negative. Acu-Rite makes several styles of these widely available thermometers. Cost \$20-25. Source: [www.weatherconnection.com](http://www.weatherconnection.com)

**Hint:** Prior to performing a test, use this thermometer to locate a consistently warm place in your home where the pouches can be incubated.

### ***Disposable Gloves***

**Feline InPouchTF cultures:** Source: [www.biomeddiagnostics.com](http://www.biomeddiagnostics.com). Cost \$5-\$7/ pouch depending on quantity purchased. An optional clip is also available to hold the pouch on the microscope for viewing the contents.

### Set-up

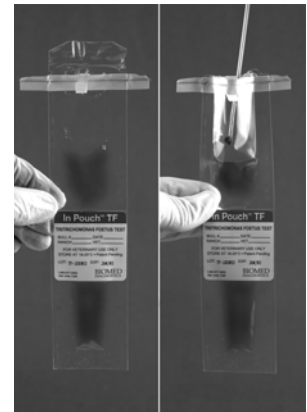
- Prior to beginning, fully acquaint yourself and any assistants with the biohazards associated with performing this test. Check to make sure all appropriate safety apparel is being worn (see Precautions above).
- Select an area where you can safely obtain the specimen and inoculate the pouch. This area should be cat-free....the last thing you need to have together is an open pouch, a fecal sample, and a curious cat.
- You should have some clean up items such as paper towels, disinfectants, and waste disposal bags available
- Label the pouch with identifying information, e.g. cat's name, date, type of specimen.

Select a method for obtaining a specimen, i.e. litter box, fecal loop or rectal swab. The latter two methods require an assistant and training by your veterinarian. Do not attempt to perform these methods without training and without an assistant. **To obtain the best test accuracy, it is strongly suggested that a fecal loop be used to obtain the test specimen.**

Obtain the specimen. How much of the specimen should be used to inoculate the pouch is always troublesome as each specimen may contain different levels of bacteria which could negatively affect the performance of the pouch. Specimens obtained by a shallow fecal loop (i.e. using a kitten loop) or by a rectal swab normally don't contain much fecal material. Specimens taken from litter boxes have an abundance of fecal material. Too much fecal material can cause a

bacterial overgrowth ruining the test so in most cases where fecal material makes up much of the specimen, less is better. If either a loop or swab is used, only mucus may be retrieved. If there is no fecal material, i.e. only mucus, the entire specimen can then be used to inoculate the pouch. If a fecal specimen is used (e.g. litter box method) limit the size of the specimen to a quantity required to form a 1/8" diameter ball. Alternatively if the stool is watery, dip a Q-tip into the puddle so that only 1/4 - 1/2 of the cotton bulb is immersed and use that amount. Also as a backup, consider inoculating two pouches for each cat being tested.

**Inoculate the pouch.** The pouch has two chambers (Fig. 1). Before opening the pouch, squeeze a small amount of liquid from the lower chamber into the upper chamber. This liquid will be used to dilute the specimen. Open the pouch by tearing (see notches in plastic) off the top while using care not to position your hand such that you will push any liquid out of the pouch. Gripping the two short white tabs on the side of the pouch, spread open the top. Insert the specimen and 'rinse off' the specimen's applicator by gently squeezing the sides of the pouch against the applicator with your thumb and forefinger. Remove the applicator and then try to remove as much air as possible while you close the pouch without spilling out the liquid. Fold (roll) the top down (like a tube of toothpaste) to force all of the liquid in the upper chamber through the center passageway to the lower chamber. While doing this try to 'burp' the pouch to prevent any air from being trapped in the lower chamber. Secure the folded portion down by folding back the wire ties.



**Incubation:** Place the pouch on edge in a light tight container (e.g. small box) and keep the temperature between 22°C (72°F) and 37°C (98°F). Keeping the pouches near or at 37°C (98°F) will reduce the time needed to see a positive result and it will significantly improve the likelihood that *T. foetus* organisms will be detected. Room temperature incubated pouches are more liable to produce false negative results.

***Hint:*** In most homes finding a way to maintain a temperature higher than room temperature without an incubator is difficult. Here are two suggestions.

1. Use a naturally and consistently warm location in the home,
2. Use a safe heating source such as a pet warmer or a reptile heater. Always monitor the pouch temperature with your thermometer to make sure the pouches remain within the recommended temperature range and never place combustible materials in contact with any heat source.

**Examining the pouch:** Remove the pouch from the container carefully so as not to disturb/mix the contents. Before placing the pouch on the microscope's stage note the clarity of the liquid media in the pouch and whether any gas bubbles are present. Unwanted growth in the pouch can cause clouding of the liquid and produce gas. Both usually occur simultaneously, but the clouding of the media appears to be the most detrimental to the validity of the test. Pouches that have become cloudy such that a microscopic examination through the clouded media is impaired should be discarded and the test repeated. The pouch should be examined daily. If *T. foetus* is present in the pouch, it is normally observable within 3-5 days. Room temperature pouches may take upwards of

12 days. To avoid missing a positive result, it is recommended that the pouch, regardless of incubation temperature, be examined daily for 12 days.

*T. foetus* organisms are not strong swimmers so they will sink to the bottom of the pouch and collect along the pouch's crease and along and amongst the settled material, e.g. feces. This area is also shallow waters so it makes *T. foetus* easier to find with the microscope.

Place the pouch on the stage of the microscope. **Do not open the pouch, the pouch's contents can be examined through the plastic.** Examine the side of the pouch where the material in the pouch has settled. A good technique is to examine the perimeter of this settled material, especially along the crease side. The settled material gives you an object on which to correctly focus your microscope. This will help you avoid focusing on the plastic surface of the pouch which may have some irregularities that could be misleading. Examine the contents of the pouch at 100X magnification. The microscope's illumination can be adjusted to suit but often a darker field increases the contrast, making the *T. foetus* easier to see.

Identifying *T. foetus* is relatively easy. After the pouch has incubated for 24 hrs the only independently moving organism in the pouch will be *T. foetus*. You'll often see a flow of the settled material in the pouch caused by the placing of the pouch on the microscope's stage, this is not *T. foetus*. This "other" material will be moving uniformly, typically multiple objects will be moving in a single direction. *T. foetus* organisms will appear like small circular or football shaped 'jitterbuggers' moving in jerky, random, non-uniform patterns.

**Note: One or more *T. foetus* organisms is a positive test result.**

A video of *T. foetus*, as observed under the microscope, can be viewed at either of the following two website locations:

[http://www.cvm.ncsu.edu/docs/jody\\_gookin.html](http://www.cvm.ncsu.edu/docs/jody_gookin.html)

[http://faculty.vetmed.ucdavis.edu/faculty/rhbondurant/t\\_foetus.mov](http://faculty.vetmed.ucdavis.edu/faculty/rhbondurant/t_foetus.mov)

A population of *T. foetus* organisms can grow until the pouch media is literally teeming with them. You may only see one or two organisms in the first few days but these will rapidly increase until every part of the pouch seems packed with them.

Verifying the infection: If a positive culture is obtained, do not discard. Your veterinarian will likely want to confirm the infection first-hand before they will be willing to prescribe treatment for your cat.

## RESEARCH ARTICLES PUBLISHED ON FELINE *T. FOETUS* INFECTION

- Gookin JL, Stauffer SH, Cocco MR, Poore MF, Levy MG, Papich MG. Efficacy of tinidazole for treatment of cats experimentally infected with *Tritrichomonas foetus*. *Am J Vet Res*; 2007;68:1085-1088.
- Gookin JL, Stauffer SH, Cocco MR, Marcotte M, Levy MG. Optimization of a species-specific PCR assay for identification of *Pentatrichomonas hominis* in canine fecal specimens. *Am J Vet Res*; 2007;68:783-787.
- Gookin JL, Stauffer SH, Levy MG. Identification of *Pentatrichomonas hominis* in feline fecal samples by polymerase chain reaction assay. *Vet Parasitol*; 2007;145:11-15.
- Dahlgren SS, Gjerde B, Pettersen HY. First record of natural *Tritrichomonas foetus* infection of the feline uterus. *J Small Anim Pract* 2007; DOI: 10.1111/j.1748-5827.2007.00405.x
- Gunn-Moore DA, McCann TM, Reed N, Simpson KE, Tennant B. Prevalence of *Tritrichomonas foetus* infection in cats with diarrhoea in the UK. *J Feline Med Surg* 2777;9:214-218.
- Kather EJ, Marks SL, Kass PH. Determination of the In Vitro Susceptibility of Feline *Tritrichomonas foetus* to 5 Antimicrobial Agents *J Vet Intern Med* 2007;21:966-970.
- Rosado TW, Specht A, Marks SL. Neurotoxicosis in 4 Cats Receiving Ronidazole *J Vet Intern Med* 2007;21:328-331.
- Gookin JL, Copple CN, Papich MG, Poore M, Stauffer SH, Birkenheuer AJ, Twedt DC, and Levy MG. Efficacy of ronidazole for treatment of feline *Tritrichomonas foetus* infection. *J Vet Intern Med* 2006; 20:536-543.
- Gookin JL. Trichomoniasis. In **Infectious Diseases of the Dog and Cat**. 3<sup>rd</sup> Ed. CE Green Editor. W.B. Saunder's Co. Philadelphia, 2006; 740-744.
- Yaeger M, Gookin JL. Histologic features of *Tritrichomonas foetus* colitis in domestic cats. *Vet Pathol*; 2005; 42: 797-804.
- Gookin JL, Birkenheuer AJ, St. John V, Spector M, and Levy M. Molecular characterization of trichomonads from feces of dogs with diarrhea. *J Parasitol*; 2005;91:939-943.
- Foster DM, Gookin JL, Poore MF, Stebbins ME, and Levy MG. Outcome of cats with diarrhea and *Tritrichomonas foetus* infection. *J Am Vet Med Assoc* 2004;225:888-892.
- Gookin JL, Stebbins ME, Hunt E, Burlone K, Fulton M, Hochel R, Talaat M, Poore M, and Levy MG. Prevalence of and risk factors for *Tritrichomonas foetus* and *Giardia* infection. *J Clin Micro* 2004;42:2707-2710.
- Gookin JL, Foster DM, Poore MF, Stebbins ME, Levy MG. Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats. *J Am Vet Med Assoc* 2003;222:1-4.
- Levy MG, Gookin JL, Poore MF, Dykstra M, Litaker RW. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline trichomonal diarrhea. *J Parasitol* 2003;89:99-104.
- Gookin JL, Birkenheuer AJ, Breitschwerdt EB, Levy MG. Single-tube nested PCR for diagnosis of *Tritrichomonas foetus* in feline feces. *J Clin Micro* 2002;40:4126-4130.
- Gookin JL, Levy MG, Law JM, Papich MG, Poore MF, Breitschwerdt EB. Experimental infection of cats with *Tritrichomonas foetus*. *Am J Vet Res* 2001;62:1690-1697.
- Levy MG, Gookin JL, Poore MF, Papich MG, Breitschwerdt EB. Intestinal trichomonosis in cats: pathology,

diagnosis and susceptibility to antiprotozoal drugs. *Proceedings of the Joint Meeting of the American Society of Parasitologists and the Society of Protozoologists* San Juan, Puerto Rico,108;2000.

Romatowski J. *Pentatrichomonas hominis* infection in four kittens. *J Am Vet Med Assoc* 2000;216:1270-1272.

Gookin JL, Breitschwerdt EB, Levy MG, Gager RB, Benrud JG. Diarrhea associated with trichomonosis in cats. *J Am Vet Med Assoc* 1999;215:1450-1455.

Romatowski J. An uncommon protozoan parasite (*Pentatrichomonas hominis*) associated with colitis in three cats. *Feline Pract* 1996;24:10-14.