

Efficacy of Ronidazole for Treatment of Feline *Tritrichomonas foetus* Infection

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Objectives: To determine the efficacy of ronidazole (RDZ), tinidazole (TDZ), and metronidazole (MDZ) against *Tritrichomonas foetus* in vitro and of RDZ for treatment of feline naturally occurring or experimentally induced *T foetus* infection.

Animals: A cat naturally infected with *T foetus* infection and diarrhea. Ten specific-pathogen-free (SPF) kittens.

Procedure: RDZ, TDZ, and MDZ were tested for activity against 3 different feline isolates of *T foetus* in vitro. RDZ then was administered to a naturally infected cat at 10 mg/kg PO q24h for 10 days. SPF kittens were infected orogastrically with feline *T foetus* and treated with either placebo or RDZ (10 mg/kg PO q12h for 14 days). Cats with relapsing infection or those receiving placebo were treated subsequently with RDZ (either 30 or 50 mg/kg PO q12h for 14 days). Feces were examined for *T foetus* by direct microscopy, culture, and polymerase chain reaction (PCR) testing weekly.

Results: Both RDZ and TDZ killed *T foetus* at concentrations >0.1 µg/mL in vitro. In the naturally infected cat, RDZ abolished diarrhea and *T foetus* infection for 85 days after treatment, at which time infection and diarrhea relapsed. Retreatment with RDZ eradicated diarrhea and *T foetus* infection for over 407 days. In experimentally induced infection, RDZ at 10 mg/kg caused initial improvement, but infection relapsed in all 5 cats 2 to 20 weeks after treatment. At 30 or 50 mg/kg, 10/10 cats were negative for *T foetus* infection for follow-up durations of 21 to 30 weeks after treatment.

Conclusions and Clinical Relevance: Oral administration of RDZ at 30 to 50 mg/kg q12h for 14 days resolved diarrhea and eradicated infection (on the basis of polymerase chain reaction [PCR] testing) in 1 naturally infected cat and 10 experimentally inoculated cats receiving a different isolate of *T foetus*.

Key words: Colitis; Diarrhea; Metronidazole; Nitroimidazole; Tinidazole.

Tritrichomonas foetus is a flagellated protozoan parasite that is best known as a venereal pathogen of cattle. Recently, *T foetus* has been identified as an enteric pathogen of domestic cats that resides within the lumen of the colon and results in colitis and chronic, foul-smelling diarrhea.^{1–6} The origin of the feline infection is unknown. The infection is spread in cats via the fecal-oral route, and dense housing is an identified risk factor for infection.^{1–3} *T foetus* infection among domestic cats in catteries has become widespread; 36/117 (31%) cats attending an international cat show were found to be infected with *T foetus*, and infection was significantly associated with a history of diarrheal disease within the cattery.⁵ An effective treatment for *T foetus* infection has yet to be identified; infected cats may have persistent diarrhea for up to 2 years, and some can remain infected for life.⁷ Trichomonads lack the ability to synthesize many essential macromolecules, which must be acquired from host secretions or by phagocytosis of host bacterial flora. Trichomonads also lack the mitochondria needed for aerobic metabolism.

Instead, pyruvate is generated by glycolysis and fermented in reductive organelles called hydrogenosomes.⁸ This reductive metabolic pathway serves as the basis for the susceptibility of trichomonads to 5-nitroimidazole antibiotics, such as metronidazole (MDZ⁸). Intact *T foetus* reduce nitroimidazoles in hydrogenosomes, which results in generation of polar autotoxic anion radicals.⁹ Feline *T foetus* infection has not been responsive to MDZ, commonly used in veterinary practice. Cats have shown transient improvement in diarrhea while receiving MDZ but remain infected with the organism.^{1,7} Because of the lack of success with MDZ, it was necessary to investigate other compounds having a similar mechanism of action but potentially greater activity against *T foetus*.

The specific aims of the present study were (1) to determine the susceptibility of feline *T foetus* to each of 3 nitroimidazole antimicrobials (ronidazole [RDZ], tinidazole [TDZ], and MDZ) in vitro and (2) to determine the ability of RDZ to resolve diarrhea and eradicate *T foetus* infection, on the basis of polymerase chain reaction (PCR) testing, when given orally to cats with either a natural or experimentally induced infection.

Materials and Methods

Isolates

Three different isolates of *T foetus* were examined for their susceptibility to RDZ, TDZ, and MDZ in vitro. Isolate 1 was derived from a naturally infected cat in a North Carolina shelter. Isolate 2 was derived from the naturally infected cat treated with RDZ in this study. Isolate 3 was derived from a cat performing in a cat show in Houston, Texas. This latter isolate was used for experimental inoculations. Each isolate was established in antibiotic-free culture medium as previously described² and positively identified as *T foetus* on the basis of single-tube nested PCR testing for detection of the ribosomal RNA (rRNA) gene unit using species-specific primers.

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In Vitro Susceptibility Assay

Feline *T foetus* in log-phase culture were inoculated at a concentration of 10^3 organisms/mL into 10-mL culture tubes containing culture medium^a supplemented with RDZ^b or TDZ^c at concentrations of 0, 0.01, 0.1, 1, and 10 $\mu\text{g/mL}$ (3 replicates each) and incubated at 37°C. For comparison, MDZ was tested at concentrations of 0, 1, and 10 $\mu\text{g/mL}$. At each concentration studied, the powder formulation of the respective nitroimidazole was dissolved directly into a volume of culture medium sufficient for aliquoting into 3 replicate tubes.

After 24, 48, and 72 hours of incubation, organisms were suspended by lightly vortexing, and then 0.1 mL of the suspended culture was diluted and fixed in 0.9 mL of 10% formaldehyde. Cell counts were performed using a hemocytometer and reported as the mean \pm SD of each triplicate dilution. At 72 hours, 1 tube at each dilution was centrifuged, and then 0.1 mL of pelleted organisms was inoculated into 10 mL of antibiotic-free medium. At 24 hours, a 10 μL aliquot of each subculture was examined by light microscopy for motile trichomonads.

The entire *in vitro* susceptibility assay was performed on each of 3 different *T foetus* isolates. Susceptibility of *T foetus* to a given concentration of drug was defined by the absence of replication over a 72-hour period and the failure of the organisms to replicate after transfer to antibiotic-free media.

Cat with Naturally Occurring Infection

A 3-year-old spayed female Persian cat was donated to North Carolina State University at 1 year of age because of a 6-month history of unrelenting large bowel diarrhea. Infection with *T foetus* was confirmed by direct microscopy, microbial culture for trichomonads,^{4,10} and single-tube nested PCR testing for detection of the rRNA gene unit of *T foetus*.¹¹ The cat was determined to be otherwise healthy on the basis of physical examination, serum biochemistry profile, preprandial and postprandial serum bile acid concentrations, feline leukemia and feline immunodeficiency virus testing, and urinalysis results. Fecal examinations also included flotation for parasite ova in saturated sodium nitrate (specific gravity 1.200); direct microscopy of a hanging drop for motile spiral organisms, such as *Helicobacter* and *Campylobacter* spp; Gram stain and antigen testing for *Giardia* spp.⁶ While housed at North Carolina State University, the cat was treated with a variety of different antimicrobials, none of which were effective in eradicating the *T foetus* infection. The cat was eventually adopted and housed alone in an outdoor facility. For the 2-year period before the present study, diarrhea remained unrelenting, often contained fresh blood and mucus, and frequently dribbled from the rectum.

A pure culture of *T foetus* was derived from the cat's feces by a method previously described,³ and the organisms were tested *in vitro* for susceptibility to RDZ, TDZ, and MDZ. The cat then was treated with RDZ at a dosage of 10 mg/kg of body weight PO q24h for 10 days. CBC, serum biochemistry profile, and urine analyses were performed immediately before and after completion of treatment. Fecal samples were collected at varying time intervals after treatment and tested with a single-tube nested PCR assay for *T foetus*. Fecal consistency was noted periodically.

Experimentally Induced Infection

Ten specific-pathogen-free (SPF) 10-week-old sexually intact female domestic shorthair cats were purchased from a commercial vendor.¹ The cats were randomly divided into 2 equal groups. Each group was housed in a separate room, and all cats in each room were kept in separate cages and received the same dry food⁶ ad libitum throughout the study. Each day, the cage liner was scraped clean and a new litter box was provided. Cats were transferred to

freshly disinfected cages once per week. Cats were housed under conditions of controlled lighting and temperature and were maintained in compliance with biosafety-level-2 guidelines. The North Carolina State University Institutional Animal Care and Use Committee approved all protocols.

During an initial 3-week acclimatization period, each cat was determined to be healthy on the basis of physical examination, CBC, serum biochemistry profile, and urinalysis results. Fecal examination for enteric pathogens included a flotation in saturated sodium nitrate (specific gravity 1.200) for parasite ova, direct microscopy for protozoa ($n = 3$ examinations/cat) and motile spiral organisms, microbial culture for trichomonads^{4, 10} (3 examinations/cat), antigen testing for *Giardia* spp,⁶ and single-tube nested PCR testing for detection of the rRNA gene unit of *T foetus*¹¹ (3 examinations/cat).

Inoculation with *T foetus*

Trichomonads were isolated from a naturally infected cat, grown in culture as previously described,³ and cryopreserved at -70°C . The organisms were thawed on ice and passed 5 times during a 2-week period before use for experimental inoculation. The final passage was established in antibiotic-free modified Diamond media,⁹ and the concentration of trichomonads was quantified by means of a hemocytometer. Each cat received 3.3 mL of media containing approximately 3×10^6 live *T foetus* via orogastric intubation. On the basis of PCR amplification and sequencing, the isolate used for experimental inoculation had 100% sequence identity with the rRNA gene sequence of *T foetus* (GenBank No. AF466749).

Treatment of Experimentally Induced Infection

Treatment with RDZ was initiated 4 weeks after the experimentally induced infection; a time period corresponding to observation of diarrhea in 100% of experimentally infected cats in a previous study.³ At that time, each group of cats ($n = 5$ each) was blindly allocated to administration of treatment or placebo. The treatment group received RDZ [(1-methyl-5-nitroimidazole-2-yl)-methyl carbamate]^b at a dosage of 10 mg/kg of body weight PO q12h. The placebo group received an equal weight of dextrose PO q12h. Both compounds were obtained from a commercial supplier^b and compounded into opaque, color-coded gel capsules by the College of Veterinary Medicine pharmacy. Each compound was administered for 14 days.

Duration of Follow-up

Feces from each cat were tested for the presence of *T foetus* on the basis of PCR testing once a week for a minimum of 6 weeks. This minimum follow-up period was originally selected based on our prior observation that the PCR assay had a sensitivity of 100% when ≥ 4 consecutive fecal samples were tested in a prior study.¹¹ Additionally, residual antibiotic theoretically could result in false-negative test results by suppressing but not eradicating *T foetus*. Thus, we conservatively estimated that residual antibiotic should be negligible after 2 weeks. After the original 6-week follow-up period, cats failing treatment at low dosages of RDZ began treatment at a higher dosage. Meanwhile, the duration of follow-up for the remaining cats was extended, resulting in recognition that cats could relapse as long as 20 weeks after completion of treatment. Thus, cats subsequently treated with 30 or 50 mg/kg of RDZ were evaluated weekly by PCR testing for a minimum of 20 weeks.

Disposition of Treatment Failures

Upon completion of the blinded study, when the cats in the treatment group had a relapsing infection or if they originally

received placebo, they were treated with RDZ at a higher dosage of either 30 mg/kg ($n = 3$) or 50 mg/kg ($n = 7$) body weight PO q12h for 14 days. Cats were tested for *T foetus* infection weekly over a minimum of 21 weeks after the last dose of RDZ. Cats receiving 10 mg/kg RDZ and failing to clear *T foetus* had their organisms re-isolated in culture and tested in vitro for susceptibility to RDZ (range 0.01 to 1000 $\mu\text{g/mL}$) followed by subculture in antibiotic-free media as previously described.

Data Collection

For cats with experimentally induced infection, fecal consistency was recorded daily on the basis of a previously published 4-point scale (1, formed [normal consistency and hard, with distinct or impacted fecal balls]; 2, semiformal [voluminous, smooth-surfaced, and soft; discrete fecal balls not evident]; 3, cow pie [not formed, consisting of loose puddles and piles of wet feces]; and 4, liquid [watery and loose].³ Also recorded daily were the total number of bowel movements, number of episodes of vomiting, and a subjective assessment of each cat's appetite and mentation (normal versus abnormal). A complete physical examination was performed once weekly.

Fecal examinations for *T foetus* were performed on all cats weekly over a 52-week period. Feces from each cat were collected from the rectum with a plastic loop and immediately processed for examination. Analysis of fecal samples for *T foetus* included microscopic examination of a fecal smear, culture of feces in a commercially available culture system,⁴ and single-tube nested PCR testing for detection of the *T foetus* rRNA gene unit using species-specific primers. These techniques have been previously described in detail.^{10,11}

Before PCR testing for *T foetus*, all samples of DNA extracted from feces were first subjected to PCR amplification of an 876 base pair (bp) gene sequence of bacterial 16S rRNA. Reaction conditions for 16S rRNA gene amplification were as follows: a 100- μL reaction volume of PCR buffer II containing 2.5 U AmpliTaq Gold DNA polymerase,⁵ 100 pmol each of primers 515F (5' GTGCCAGCAGCCGCGGTAA 3') and 1391R (5' GACGGG-CGGTGAGTGCA 3'), 200 μM each deoxynucleotide triphosphate, 10 μg of bovine serum albumin, and 5 μL of DNA template. DNA amplification was performed at the following temperature profiles: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 48°C for 1 minute, and extension at 72°C for 2 minutes for 50 cycles, followed by a final extension for 5 minutes at 72°C. By performing PCR testing for bacterial 16S rRNA gene, the possibility that a negative PCR test result for *T foetus* could be attributed to the presence of endogenous PCR inhibitors in the extracted DNA was ruled out for each sample.

For evaluation of adverse acute systemic effects, CBC, serum biochemistry profile, and urine analyses were performed on all cats immediately before experimental inoculation with *T foetus* and within a week after completion of treatment with placebo or RDZ (10 mg/kg). Prior studies with this model have shown that infection alone does not result in hematologic or serum biochemical abnormalities.³ In addition, all 7 cats treated with RDZ at 50 mg/kg had CBC, serum biochemistry profile, and urine analyses performed immediately before and within a week after completing treatment.

Statistical Analysis

All statistical analyses were performed using a commercial software package.¹ To determine whether *T foetus* infection significantly increased fecal consistency scores or defecation frequency compared to the pre-infection period, the daily fecal consistency score and number of bowel movements of each

individual cat were each averaged over the 2-week period before inoculation with *T foetus* and over the 2-week period after inoculation with *T foetus*. Whether there was a difference in average fecal consistency score or defecation frequency of cats between the pre-infection and postinfection periods was tested using a Student's paired *t*-test. For all analyses, values of $P < .05$ were considered significant.

Results

In Vitro Susceptibility of *T foetus* to 5-Nitroimidazoles

There was no inhibitory effect with MDZ on proliferation of *T foetus* in vitro at concentrations ≤ 10 $\mu\text{g/mL}$. Both TDZ and RDZ arrested growth of *T foetus* in vitro at concentrations ≥ 0.1 $\mu\text{g/mL}$. At a concentration of 0.01 $\mu\text{g/mL}$, more sustained attenuation of growth was observed with RDZ (Fig 1). Trichomonads did not replicate after transfer from tubes containing ≥ 0.1 $\mu\text{g/mL}$ TDZ or RDZ to antibiotic-free media.

Effect of RDZ on Naturally Occurring Infection in a Cat

Within 24 hours of initiating treatment with RDZ at 10 mg/kg PO q24h, trichomonads were no longer detected by direct examination of fecal smears. Improvement in fecal consistency was immediate and dramatic, with feces returning to normal after completion of the 10-day treatment course (Fig 2). No adverse effects, such as vomiting or inappetence, were observed during or after treatment. Results of CBC, serum biochemistry profile, and urine analyses remained within reference range limits. Feces remained formed and tested negative for *T foetus* infection by culture and single-tube nested PCR assay on days 15 and 31 after treatment. On day 85 after treatment, there was an acute onset of large bowel diarrhea containing fresh blood and mucus. Feces were again positive for *T foetus* infection by direct smear examination, culture, and single-tube nested PCR testing (Fig 2). Diarrhea was so severe that perineal soiling with feces resulted in infestation by maggots, which was treated by local debridement, wet-to-dry bandaging, and administration of systemic antibiotics.

T foetus was re-isolated from the cat's feces and was determined in vitro to remain susceptible to killing by RDZ at concentrations ≥ 0.1 $\mu\text{g/mL}$. The cat was retreated with RDZ at the same dosage and duration (10 mg/kg body weight PO q24h for 10 days). Immediate resolution of diarrhea and fecal shedding of trichomonads again was observed. Feces remained formed, and the cat has tested negative for *T foetus* infection by single-tube nested PCR assay on days 22, 37, and 407 after retreatment with RDZ (Fig 3).

Experimentally Induced Infection with *T foetus*

No cat tested positive for *T foetus* before the experimentally induced infection. Feces from 1 cat contained small numbers of *Isospora* spp ova. Within 2 weeks of orogastric inoculation, feces from each cat tested positive for *T foetus* infection. There was

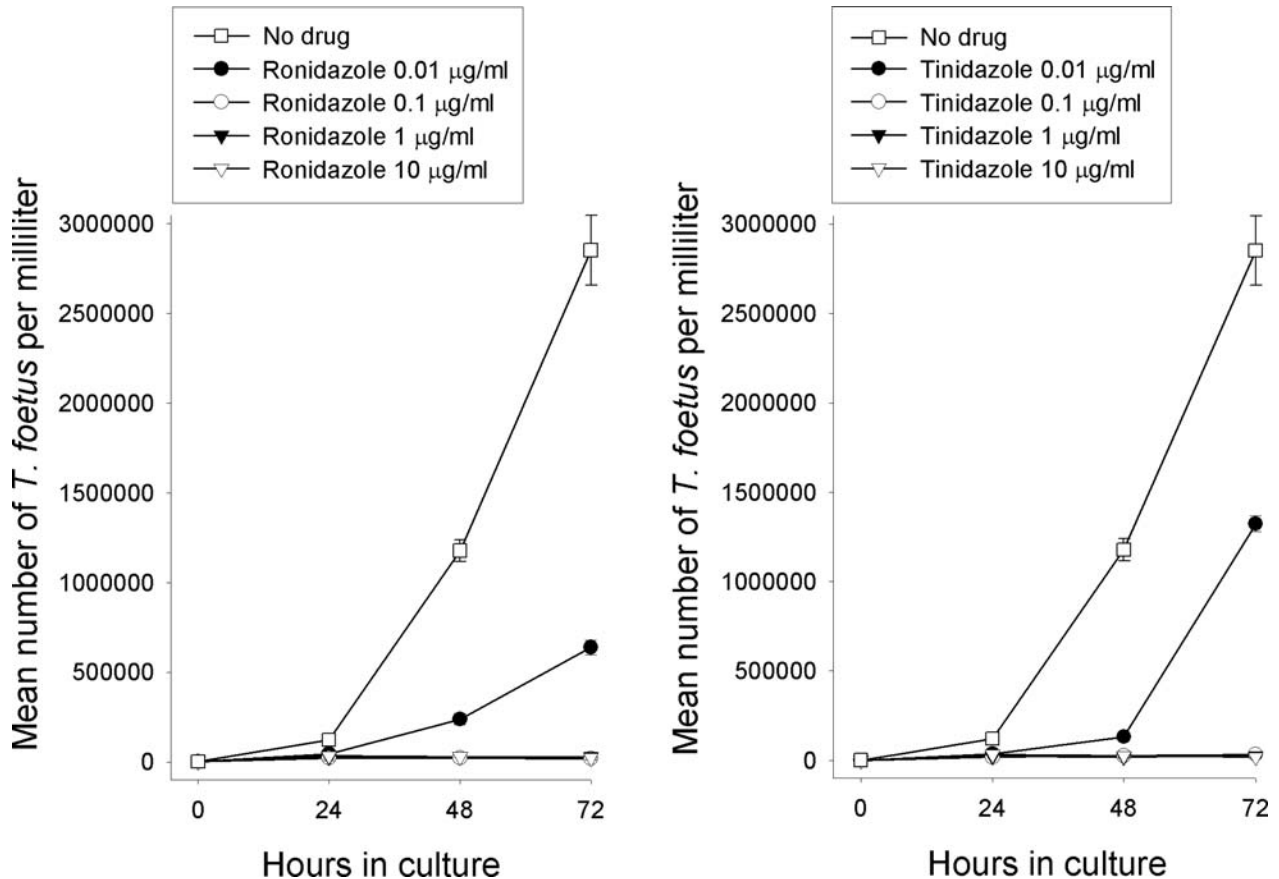


Fig 1. In vitro susceptibility of feline *Trichostrongylus axei* to increasing concentrations of ronidazole (RDZ) or tinidazole (TDZ). Data points represent the mean \pm SD of 3 replicates at each dilution. Data shown are from one isolate and are representative of 3 separate experiments, each performed with a different isolate of feline *T. foetus*.

a significant difference in the average fecal consistency of cats after infection compared to the pre-infection period ($P = .01$, Fig 3). All cats developed semiformal to liquid diarrhea by the 2nd week of infection. In 6 cats, involuntary dribbling of feces also was observed (Fig 3). There was no difference in the frequency of defecation after infection with *T. foetus* (average number of bowel movements per day: 1.2 ± 0.22 before infection, 1.3 ± 0.29 after infection [$n = 10$, $P = 0.12$]). Despite persistent infection, severity of diarrhea began to abate in all cats before beginning treatment with RDZ (Fig 3). Therefore, it was not possible to statistically evaluate whether treatment with RDZ improved fecal consistency. Spontaneous resolution of *T. foetus* infection was not observed in any cat.

Effect of RDZ on Cats with Experimentally Induced Infection

All 5 cats in the treatment group receiving RDZ at 10 mg/kg of body weight PO q12h became negative for *T. foetus* infection (as determined by direct fecal smear, culture, and PCR testing) within 3 days of initiating treatment. These cats remained negative throughout the 2-week treatment period and for 1 week thereafter. However, a relapsing infection was detected at least once in each cat in the treatment group at 2, 3, 3, 17, or 20

weeks after the treatment course was completed (Fig 4). *T. foetus* was re-isolated from 1 of these cats' feces and was determined in vitro to remain susceptible to killing by RDZ at concentrations $\geq 0.1 \mu\text{g/mL}$. The 5 cats receiving placebo remained positive for *T. foetus* infection throughout this time.

Three treatment group cats (those relapsing at 2 or 3 weeks after treatment) were retreated with RDZ at 30 mg/kg of body weight PO q12h for 14 days. The remaining 2 treatment group cats (those relapsing at 17 and 20 weeks after treatment) and those originally treated with placebo ($n = 5$) were treated with RDZ at 50 mg/kg of body weight PO q12h for 14 days. Two cats receiving 30 mg/kg and all 7 cats receiving 50 mg/kg remained negative for *T. foetus* for follow-up durations of 30 and 21–23 weeks after treatment, respectively (Fig 4). The remaining cat that received 30 mg/kg RDZ also was negative for *T. foetus* for 18 weeks after treatment, at which time the cat died acutely. Complete postmortem and histopathologic examinations did not identify a cause of death.

Adverse events (eg, lethargy, vomiting, anorexia) were not observed during or after treatment with RDZ. Subjectively, feces were softer while cats received RDZ at 50 mg/kg of body weight. Results of CBC, serum biochemistry profile and urine analyses performed before and immediately after cats received

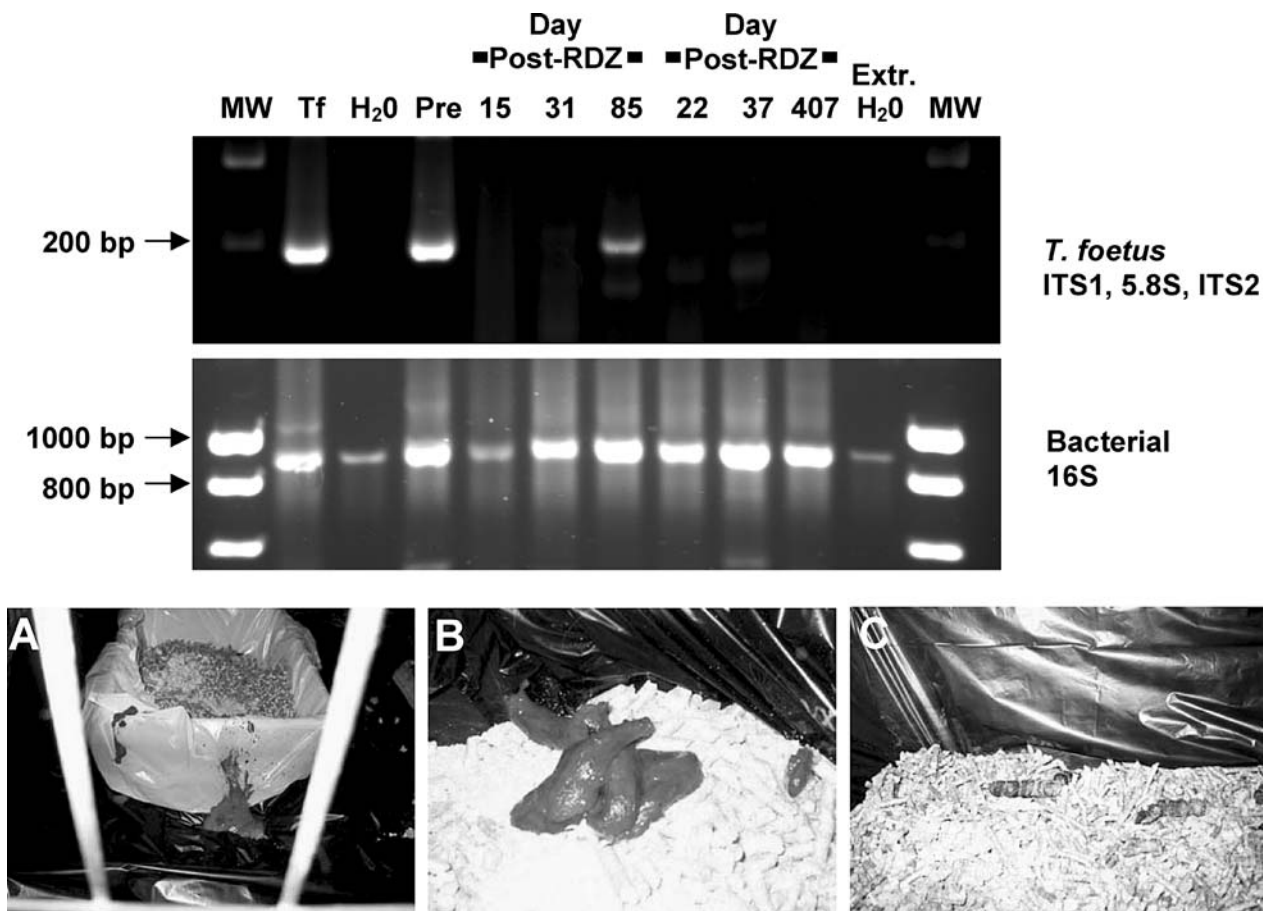


Fig 2. Analysis of single-tube nested polymerase chain reaction (PCR) amplification products of *Trichostrongylus axei* partial rRNA gene unit by 1.5% agarose gel electrophoresis followed by staining with ethidium bromide. From left to right, lanes show the following: molecular weight markers; *T. foetus* genomic DNA, sterile water (PCR contamination control); DNA extracted from feces of a naturally infected cat before (day 0 = pre) and after treatment with RDZ (day 15 and 31) and at the time of relapse infection (day 85); DNA extracted from feces of a naturally infected cat 22, 37, and 407 days after retreatment with RDZ; DNA extracted in the absence of feces (DNA extraction contamination control); and molecular weight markers. Analysis of bacterial 16S rRNA gene products in each sample ruled out the presence of endogenous PCR inhibitors. Appearance of voided feces from a cat with naturally occurring *T. foetus* infection: Panel A, before treatment with RDZ; Panel B, after 48 hours of treatment with RDZ; and Panel C, 2 months after completion of treatment with RDZ.

RDZ at 10 mg/kg or 50 mg/kg body weight remained within reference range limits. Because clinically relevant changes between pretreatment and posttreatment hematologic or biochemical data were not observed, statistical comparisons were not performed.

Discussion

An effective treatment for feline *T. foetus* infection or the associated diarrhea has not been reported previously. In infected cats, diarrhea typically waxes and wanes, often appearing to respond to treatment only to relapse shortly after treatment is discontinued.¹ MDZ often is chosen as initial therapy, either because trichomonads initially are mistaken for *Giardia* spp. or are thought to be MDZ-sensitive trichomonads, such as *Pentatrichomonas hominis*, or because no other proven treatment options exist. Our finding in the present study that MDZ had no activity against 3 different isolates of *T. foetus* in vitro is consistent with the observed lack of its clinical efficacy.¹

In the present study, we tested 2 related 5-nitroimidazoles, TDZ and RDZ, for in vitro activity against feline *T. foetus*. TDZ is licensed for use in people for treatment of infections caused by MDZ-resistant *Trichomonas vaginalis*. RDZ has demonstrated activity against *Histomonas meleagridis* (turkey blackhead), *Treponema hyodysenteriae* (swine dysentery), and *Trichomonas gallinae* infection (pigeon canker).¹³⁻¹⁵ RDZ is not registered for human or veterinary use in the United States, but chemical grade RDZ is commercially available.^b

In vitro, RDZ and TDZ demonstrated microbicidal activity against 3 different isolates of feline *T. foetus*. The activity of RDZ was slightly greater than that of TDZ. Thus, RDZ was chosen for evaluation in treating in vivo *T. foetus* infection. The dosage of RDZ for in vivo treatment of *T. foetus*-infected cats was extrapolated from the dosing recommendations for the related nitroimidazole MDZ,¹⁶ the dosage reported for use in pigeons,¹⁵ and the results of empiric treatment of the naturally infected cat in this report. Although there are

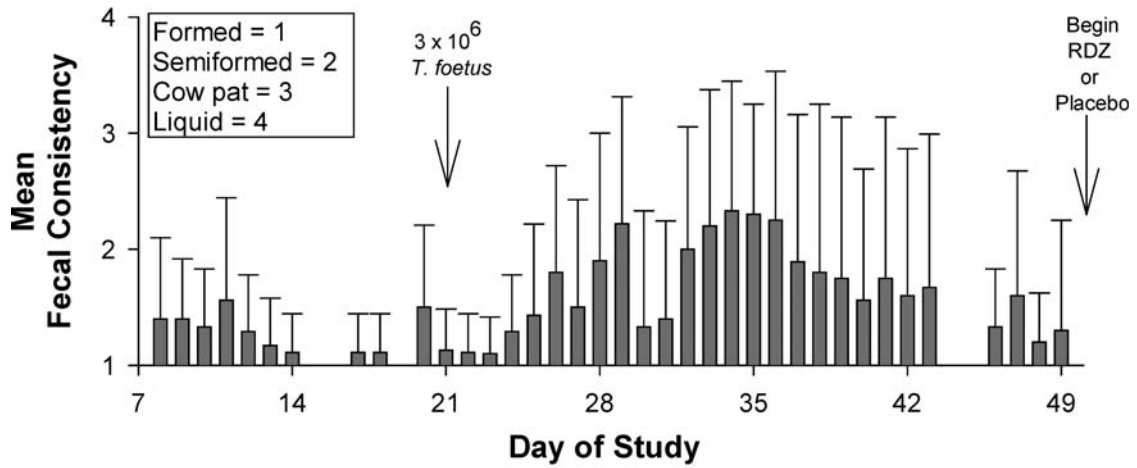


Fig 3. Daily mean (\pm SD) fecal consistency score for 10 cats before and after experimental inoculation with *Tritrichomonas foetus*. Fecal consistency for each cat was scored daily as follows: 1, formed; 2, semiformed; 3, cow pie; and 4, liquid. Ronidazole (RDZ) administered at 10 mg/kg PO q12h. Appearance of voided feces and perineal region of a cat with experimentally induced *T. foetus* infection. Note the severity of diarrhea (score = 4) and the presence of fecal incontinence.

no pharmacokinetic or toxicity data on the use of RDZ in cats, RDZ has a wide therapeutic safety margin in birds.¹⁷ Furthermore, *T. foetus* has been shown to avidly metabolize RDZ by pathways different from those used by mammalian and gut bacterial enzymes, which may provide attributes of safety for RDZ compared to MDZ.¹⁸

In the present study, RDZ was well tolerated by all cats receiving dosages up to 50 mg/kg of body weight q12h for 14 days. When administered to a naturally infected cat, RDZ resulted in acute, marked, and sustained resolution of diarrhea, despite a prolonged history of unrelenting large bowel diarrhea. Unfortunately, we were unable to evaluate the effect of RDZ on

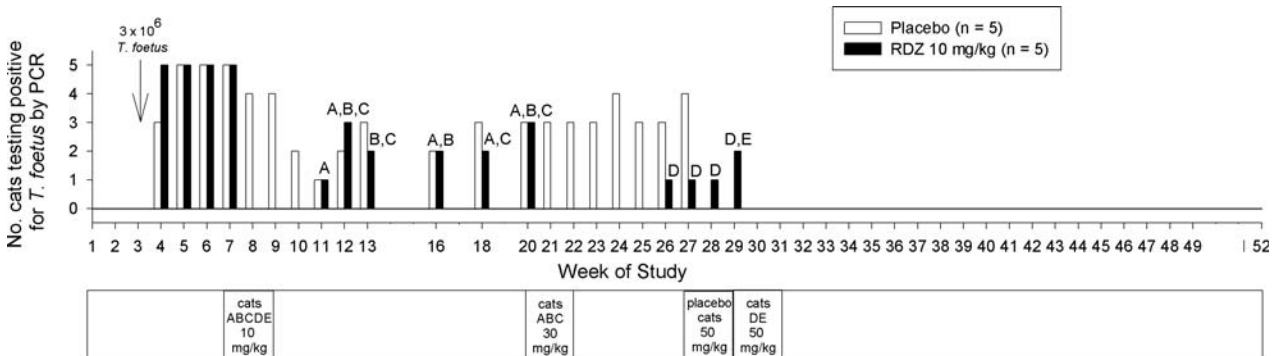


Fig 4. Time line of experimental feline *Tritrichomonas foetus* infection and response to treatment with ronidazole (RDZ). Ten specific-pathogen-free cats were acclimated for 3 weeks before experimental infection with *T. foetus* (arrow). Before and after infection, feces from each cat was tested on the weeks shown for the presence of *T. foetus* by a single-tube nested PCR assay. Cats originally receiving 10 mg/kg RDZ are designated as A, B, C, D, and E. Onset and duration of each treatment period is shown beneath the x-axis, and indicates the dose administered and identity of the cats receiving each dose.

diarrhea in experimentally infected cats because diarrhea began to spontaneously abate by the time treatment was initiated 4 weeks after infection. In response to treatment with RDZ, all cats became negative for *T foetus* on the basis of direct smear, culture, and single-tube nested PCR testing of fecal samples. However, infection relapsed in all cats receiving RDZ at a dosage of 10 mg/kg body weight. Relapse caused by suboptimal dosage rather than re-infection is considered more likely in these cats because all were housed separately and with strict attention to sanitation. *T foetus* that was recultured from the feces of 1 cat with relapsing infection remained susceptible to killing by RDZ in vitro. At RDZ doses of 30 or 50 mg/kg body weight, all cats became negative for *T foetus* on the basis of repeated PCR testing over durations of 21 to 30 weeks. We believe that the spontaneous remission of *T foetus* infection in the cats reported here is unlikely. First, spontaneous remission of *T foetus* infection was not observed in any cats in this study. Second, spontaneous remission was not observed in any cats over a period of ≥ 50 weeks after experimental infection with *T foetus* in a prior study.³ Finally, all cats became PCR negative for *T foetus* infection within days of initiating treatment with RDZ.

Compared to MDZ, the greater efficacy of RDZ for treatment of cats with *T foetus* infection may be attributed to a higher activity of RDZ in vivo and trapping of the activated compound within the intestine. RDZ has a 10-fold higher in vivo activity against trichomonal infections compared to MDZ.¹⁸ In pigs, a high fraction of RDZ is found in the feces, which may be the result of reductive activation of the compound and trapping of the reactive intermediate by anaerobic organisms in the intestinal tract.¹⁹

In the present study, a sensitive and specific PCR assay was used to repeatedly test the feces of cats for the presence of the rRNA genes of *T foetus*. This PCR assay has been validated for use with DNA extracted from feline feces and is the only PCR assay superior to culture for demonstrating *T foetus* organisms in biological samples.^{5,20-22} Feces is considered to be one of the most complex biological samples for PCR testing because of the presence of inherent PCR inhibitors, such as heme, bilirubin, bile salts, and complex carbohydrates.²³ To minimize the possibility of false-negative results caused by the presence of PCR inhibitors, DNA extractions were performed by a protocol optimized for fecal samples,¹¹ and every DNA sample was tested for PCR inhibitors by a separate reaction in which bacterial 16S rRNA genes were amplified before testing with *T foetus*-specific primers. The ~ 900 bp 16S rRNA gene amplicon was frequently detected in negative control samples (Fig 2), which is consistent with the presence of low amounts of bacterial DNA in Taq polymerase.²⁴ Because the purpose of this PCR test was to detect the presence of PCR inhibitors in each sample before testing for *T foetus*, we decided not to pretreat the Taq polymerase with DNase I because this procedure has been associated with reduced sensitivity of subsequent PCR.²⁴

Our findings suggest that RDZ at 30 to 50 mg/kg body weight PO q12h for 14 days results in long-term

elimination of *T foetus* infection and diarrhea in the cat. Nevertheless, although PCR testing can be used to confirm infection, it cannot be used to conclusively prove that infection has been eradicated. In the present study, prolonged periods of asymptomatic, PCR-negative intervals were observed after initial therapy with RDZ. Our duration of follow-up was extended from 6 to ≥ 21 weeks when we observed that infection could relapse as long as 20 weeks after treatment. Thus, short-term studies of drug efficacy in *T foetus* infected cats should be viewed with caution. In all cats that relapsed after initial treatment with RDZ, follow-up treatment with RDZ eradicated infection.

In the present study, 3 different strains of feline *T foetus* were demonstrated to be susceptible to killing by RDZ. At least 1 of these strains is known to have been derived from a cat previously treated, without success, with multiple antimicrobial drugs. In practice, the efficacy of RDZ may be altered by variation in antimicrobial susceptibility among different field strains of feline *T foetus*. Thus, clinical experience ultimately will be necessary to determine whether RDZ cures *T foetus* infected cats and eliminates them as carriers for transmission.

Footnotes

^a Modified Diamond medium, Remel, Lenexa, KS

^b Ronidazole [(1-methyl-5-nitroimidazole-2-yl)-methyl carbamate; >99% pure] R7635-5G, Sigma Chemical Co, St. Louis, MO

^c Tinidazole [1-(2(ethylsulfonyl)ethyl)-2-methyl-5 nitroimidazole] T3021, Sigma Chemical Co, St. Louis, MO

^d In Pouch TF, Biomed Diagnostics Inc, White City, OR

^e ProSpecT *Giardia* Rapid Assay, Alexon-Trend Inc, Ramsey, MN

^f Liberty Research Inc, Waverly, NY

^g Hills' Science Diet Feline Growth, Hills' Nutrition, Topeka, KS

^h Perkin-Elmer, Foster City, CA

ⁱ SigmaStat statistical software, Jandel Scientific, San Rafael, CA

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