

An evaluation of the use of ronidazole for the treatment of *Tritrichomonas foetus* in cats

A Knowledge Summary by

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ISSN: 2396-9776 Published: 04 Dec 2019 in: Vol 4, Issue 4 DOI: <u>10.18849/VE.V4I4.263</u> Reviewed by: Katherine Briscoe (BVSc (Hons I), MVetStud, FANZCVS) and Nicolette Joosting (BSc (Hons),

Next Review Date: 03 Mar 2021



KNOWLEDGE SUMMARY

PICO question

In cats infected with *Tritrichomonas foetus*, does treatment with oral ronidazole compared to an alternative antiprotozoal treatment or placebo result in successful resolution of clinical signs and eradication of disease?

Clinical bottom line

Ronidazole use appears to be efficacious in eradicating infection with *Tritrichomonas foetus* and resolving diarrhoea associated with infection. A dose range of 30–50 mg/kg 12–24 hourly has been suggested, with evidence suggesting that a dose of 30 mg/kg 24 hourly for 14 days may be effective. However, some cats may require higher doses and some may not respond to treatment, and relapse may occur during a protracted period following completion of the treatment course. Neurological side effects appear to be uncommon but may occur with doses of 30 mg/kg and above.

A total of six studies are reviewed: Three randomised, controlled studies, one cohort study and two case series (one retrospective). Findings indicate efficacy of ronidazole treatment in eradicating infection and resolving diarrhoea, however many studies involved small sample sizes and limited follow-up. Therefore, evidence to support the use of ronidazole in *Tritrichomonas foetus* infected cats remains relatively limited.

Clinical Scenario

Tritrichomonas foetus is one of the most common causes of infectious colitis in cats. Remission from the diarrhoea associated with *T. foetus* infection may occur with time, however persistence of infection is common. This is particularly pertinent in cattery and shelter populations in which *T. foetus* infection is widespread. Ronidazole is currently the only drug with demonstrated efficacy against *T. foetus*, however it has a narrow safety margin and its use is currently off-license in the UK (Gookin et al. 2017).

The evidence

There is a small body of *in vivo* evidence for the treatment of cats infected with *Tritrichomonas foetus* using ronidazole, and of that evidence only half of the relevant papers are randomised, controlled, blinded studies. Therefore, the strength of the evidence is relatively low. A significant proportion of the evidence is from case series or cohort studies, where bias may be inherently introduced from case selection, the lack of blinding, no presence of a control/comparator and loss of patients to follow-up.

Most studies identified had a small sample size, with a limited follow up period in many.

There are three randomised, controlled studies examining the efficacy of ronidazole for treatment of *T. foetus* infection compared to placebo, all utilising different doses (10–50 mg/kg) and different dosing schedules (12–24 hourly).



Summary of the evidence

Grellet et al. (2017)	
	Cate water with infected with T factors in French action
Population:	Cats naturally infected with <i>1. joetus</i> in French catteries
Sample size:	47 cats
Intervention details:	 Pharmacokinetics of ronidazole determined <i>in vitro</i> prior to the clinical study being undertaken. Existing infection with <i>T. foetus</i> detected via polymerase chain reaction (PCR) undertaken on rectal swab sample. Confirmed infected cats randomly allocated into two groups: placebo (n= 22) or treatment (n= 25). Treatment group received ronidazole at 30 mg/kg once daily for 14 days in guar gum coated capsule formulation, placebo received capsule without ronidazole.
Study design:	Randomised, controlled, double-blinded study
Outcome studied:	 Repeat rectal swab undertaken after 14 days treatment, repeat PCR undertaken. Cats deemed still infected if PCR detected <i>T. foetus</i> presence (objective assessment).
	 Clinical examination undertaken and presence of adverse effects recorded (subjective assessment).
Main findings: (relevant to PICO question):	 Treatment group – 84% (21/25) <i>T. foetus</i> not detected in cats that received ronidazole. Placebo group – 18% (4/22) <i>T. foetus</i> not detected in cats that received placebo. Statistically significant difference between treatment and placebo groups (P <0.001). No adverse drug reactions observed.
Limitations:	 Adverse effects of treatment are mentioned in the introduction, and the authors have stated that any 'adverse effects' were recorded; however, they do not describe which specific adverse effects were being screened for in methods. Dose of ronidazole administered is alluded to in the introduction, but there is no explanation in the method section as to why the specific dose/dosing schedule used in this study was selected. Final PCR undertaken at end of 14 day treatment course, no longer-term follow-up once treatment stopped. 4/25 (16%) of treated cats were still infected at the end of the study, however cats appear to have not been kept separately during the treatment phase and were in a multi-cat scenario. Therefore it is difficult to know whether these cats were still infected due to treatment failure, resistance of <i>T. foetus</i> to ronidazole or reinfection.

Gookin et al. (2006)	
Population:	An adult cat with naturally occurring <i>T. foetus</i> infection and 10 week old, specific-pathogen free kittens
Sample size:	 One adult cat with naturally acquired infection 10 specific-pathogen free kittens
Intervention details:	 Susceptibility of <i>T. foetus</i> to ronidazole, tinidazole and metronidazole determined <i>in vitro</i> prior to clinical study. Infection with <i>T. foetus</i> confirmed in adult cat via direct microscopy, faecal culture and PCR on faecal sample. Organisms obtained to use in <i>in vitro</i> susceptibility study. Adult cat treated with 10 mg/kg ronidazole q 24 hours for 10 days. Specific-pathogen free kittens randomly split into two equal groups (n=5). Health determined during initial 3 week acclimatisation period via clinical examination, biochemistry, haematology and urinalysis. Faeces examined for enteric pathogens via faecal flotation, direct microscopy, microbial culture, antigen testing for <i>Giardia spp.</i> and PCR for detection of <i>T. foetus</i> ribonucleic acid (RNA) Kittens experimentally infected via orogastric intubation with <i>T. foetus</i> in specifically prepared medium containing approximately 3 x 10⁶ live <i>T. foetus</i>. Treatment initiated 4 weeks after experimentally induced infection. Kittens received either ronidazole (10 mg/kg) or dextrose (placebo) twice daily q 12 hours via colour coded gel capsules for 14 days.
Study design:	Randomised, controlled study (specific-pathogen free kittens)
Outcome studied:	 PCR undertaken on faeces weekly for 6 weeks to determine presence of <i>T. foetus</i> (objective). Cats with residual <i>T. foetus</i> still present given treatment with higher dose ronidazole 30 or 50 mg/kg q 12 hours for 14 days. Weekly PCR undertaken on faeces for minimum of 20 weeks. Presence of adverse effects (subjective).
Main findings: (relevant to PICO question):	 Adult cat treatment group: No trichomonads on faecal screen after 24 hours treatment. Immediate improvement in faecal consistency, normal after 10 day treatment course. <i>T. foetus</i> not identified via PCR at day 15 or 31 after treatment. Adult cat day 85 post-treatment: Relapse of clinical signs and repeat isolation of <i>T. foetus</i>, resolved with repeat treatment using ronidazole at same dose and duration. No further isolation of <i>T. foetus</i> up to day 407 after treatment. Kitten treatment group: Resolution of <i>T. foetus</i> following treatment, relapse of infection in all kittens in follow-up period after completion of treatment (2–20 weeks).

	 Kitten placebo group: all remained positive. All relapsed and placebo-treated cats were re-treated with either 30 or 50 mg/kg ronidazole q 12 hours for 14 days. Resolution of infection documented. No relapse identified in follow-up period of 30 and 21–23 weeks respectively. No adverse events reported during or after treatment in all groups. Subjectively softer faeces were reported in cats while receiving 50 mg/kg ronidazole.
Limitations:	 Unclear why the single naturally infected cat was included in the study. Small sample size of 10 specific-pathogen free kittens, no power calculation included. Study not fully blinded as colour coded capsules used to differentiate those containing ronidazole from placebo. Unclear why a low dose of ronidazole was initially utilised, then two different higher doses (30 mg/kg or 50 mg/kg) were used when relapse occurred or to treat infected kittens originally administered with the placebo.

Gookin et al. (2010)	
Population:	Abyssinian cats in a cattery
Sample size:	11 cats
Intervention details:	 8/11 cats in a single cattery with diarrhoea and positive diagnosis of <i>T. foetus</i> on faecal culture. Treatment with 35–45 mg/kg ronidazole q 12 hourly for 14 days (1–2 courses). Repeat faecal cultures (2–5 cultures per cat).
Study design:	Case series
Outcome studied:	 Resolution of diarrhoea (subjective) Faecal culture for presence of <i>T. foetus</i> (objective) <i>In vitro</i> susceptibility to ronidazole in aerobic and anaerobic conditions (objective).
Main findings: (relevant to PICO question):	 Treatment with one (seven cats) or two (one cat) courses of ronidazole 35–45 mg/kg q 12 hourly for 14 days resulted in repeatedly negative faecal cultures for <i>T. foetus</i> in six cats and resolution of diarrhoea. Infection was still present in 2/8 cats. Repeat courses of metronidazole (20–32 mg/kg q 24 hours, unknown duration), tinidazole (50 mg/kg q 24 hours for 14 days) and ronidazole (35–60 mg/kg q 12 hours for 14 days) were administered to these cats over the course of 1 year. Multiple courses of metronidazole, tinidazole and ronidazole did not clear <i>T. foetus</i> infection suggesting <i>in vivo</i> resistance. After 1 year of treatment, infection was still not eradicated in 2/8 cats. Faeces from these two cats was collected and <i>in vitro</i> susceptibility to ronidazole tested.

	 In vitro resistance of <i>T. foetus</i> to ronidazole demonstrated in aerobic conditions. No weight loss, inappetence or neurological signs observed. One cat vomited three times in first 24 hours of treatment although unclear which drug was being administered at this point.
Limitations:	 In vivo part of study not controlled or blinded. Small sample size. Resistance of <i>T. foetus</i> to ronidazole <i>in vivo</i> assumed from non-response to treatment, however small cohort examined (n=2). Cats included in study were blanket treated with different drug courses/combinations rather than a defined treatment regimen. Susceptibility of <i>T. foetus</i> to ronidazole in aerobic conditions suggested in this study, however this was only demonstrated in one cat. Further studies required to substantiate this claim.

Koster et al. (2015)	
Population:	Medical records of domestic cats presented to two veterinary clinics in Hong Kong over a 5 year period. Only cases with complete medical record and confirmed <i>T. foetus</i> diagnosis (history, clinical examination and details of diagnostic testing) included.
Sample size:	29 cats
Intervention details:	 Diagnosis of <i>T. foetus</i> made by combination of faecal smear microscopy and faecal PCR. All cats treated with 30 mg/kg ronidazole q 24 hourly for 14 days.
Study design:	Case series, retrospective
Outcome studied:	 Signalment of affected cats (objective). Presence of diarrhoea (subjective). Response to ronidazole treatment at recommended dosing schedule (objective). Presence of co-infection with <i>Giardia spp.</i> (objective).
Main findings: (relevant to PICO question):	 25/29 (86%) of cats were purebred, 19/29 (66%) were male, median age 10 months. All 29 cats had diarrhea identified in the medical record. 13 had large bowel diarrhoea, 16 had mixed small- and large-bowel diarrhoea. 24/29 (83%) cats responded to ronidazole treatment, with resolution of clinical signs. 5/29 (17%) cats did not respond, two of which were from multi-cat households. 9/29 (31%) cats were co-infected with <i>Giardia spp</i>.
Limitations:	 Retrospective study of medical records, therefore lack of control group and not, randomised or blinded. Bias in signalment may be present as owned cats presenting to



Lim et al. (2012)	
Population:	5–7 month old Korean Domestic Short Hair kittens
Sample size:	Six kittens
Intervention details:	 Kittens confirmed free of <i>T. foetus</i> via faecal smear, culture from rectal swab and PCR. Kittens divided into two groups (n=3), sedated and administered media containing <i>T. foetus</i> trophozoites via feeding tube. Infection with <i>T. foetus</i> confimed via faecal examination of trophozoites by day 20 post inoculation. Treatment group (n=3): Oral administration of 50 mg/kg ronidazole in gel capsules q 12 hourly for 14 days Placebo group (n=3): Empty gel capsule placebo from day 30 post inoculation. Faecal smear, culture from rectal swab and PCR undertaken once weekly for following 4 weeks.
Study design:	Randomised controlled, non-blinded study
Outcome studied:	 Presence of diarrhoea following experimental infection (subjective).
	 Presence of <i>T. foetus</i> in faeces during treatment period and 4 week follow-up (objective).
Main findings: (relevant to PICO question):	 Presence of <i>T. foetus</i> in faeces during treatment period and 4 week follow-up (objective). No diarrhoea was observed in any cat following experimental infection. Treatment group: All cats negative for <i>T. foetus</i> during treatment period and 4 week follow-up period. Placebo group: All cats remained positive for <i>T. foetus</i> throughout the study.



Short-term follow-up period of 4 weeks, treatment appears
effective during the immediate post-treatment period, however
it cannot be determined whether ronidazole administration at
this dose is effective at preventing relapse over longer periods.

Reinert et al. (2016)	
Population:	Abyssinian cats. Two male, five female cats with intermittent large bowel diarrhoea despite treatment with fenbendazole.
Sample size:	Seven cats
Intervention details:	 <i>T. foetus</i> diagnosed via faecal PCR. Five remaining cats treated with 30 mg/kg ronidazole q 24 hourly for 14 days. Cats kept separately in isolation and given physical examinations and neurological examinations daily. Repeat PCR undertaken on faeces over period of 345–800 days. Two cats lost through euthanasia as result of other conditions. Cats euthanised following <i>T. foetus</i> diagnosis but prior to starting treatment with ronidazole.
Study design:	Cohort study
Outcome studied:	 Presence of <i>T. foetus</i> in faeces via PCR in follow-up period (objective). Presence of side effects of treatment (subjective).
Main findings: (relevant to PICO question):	 Neurological side effects seen in two cats, treatment paused for one day then restarted. No evidence of <i>T. foetus</i> (via faecal PCR) in any cat during up to 800 day follow-up, infection deemed eliminated. 2/7 cats initially diagnosed with <i>T. foetus</i> lost to follow up due to euthanasia.
Limitations:	 No discussion about presence/improvement of diarrhoea over follow-up period. No discussion as to why ronidazole dose chosen was used. Small sample size. Cohort study, no control/comparator group.

Appraisal, application and reflection

There are few papers examining the *in vivo* efficacy of oral ronidazole treatment in cats infected with *Tritrichomonas foetus*. Of these papers, half were randomised, controlled, blinded studies comparing the use of oral ronidazole with placebo, however the remaining half were case series or cohort studies. Therefore, there is a significant amount of lower quality evidence present.



One major problem present in most of the papers is that a small sample size was utilised. Although many results were significant, this may mean that the results are not representative of a wider population and conclusions may not be robust.

Infection with *T. foetus* was induced experimentally via orogastric intubation in two of the randomised, controlled studies (Gookin et al. 2006 and Lim et al. 2012), therefore the results of these studies may not be truly reflective of naturally occurring infection.

There is significant variation in the treatment protocols described in the studies. All three randomised, controlled, blinded studies utilised different doses and dosing schedules of ronidazole. Grellet et al. (2017) used 30 mg/kg once daily for 14 days, Gookin et al. (2006) initially used 10 mg/kg twice daily for 14 days, then 30–50 mg/kg twice daily for 14 days and Lim et al. (2012) used 50 mg/kg twice daily for 14 days.

Both Grellet et al. (2017) and Lim et al. (2012) observed eradication of *T. foetus* in treated cats at the end of treatment after 14 days of treatment (determined via PCR on rectal swab), however clinical signs of diarrhoea were not examined and there was limited monitoring for recurrence following cessation of treatment. Gookin et al. (2006) observed that lower dose (10 mg/kg) ronidazole did initially resolve infection during the treatment period, however all kittens relapsed within 2–20 weeks and required further treatment with 30–50 mg/kg ronidazole twice daily, after which no relapse was observed in the following 21–30 weeks and diarrhoea resolved.

The use of 35–45 mg/kg ronidazole twice daily for 14 days was also demonstrated to eradicate *T. foetus* on faecal culture and resolve diarrhoea in six cats, with repeated negative culture on follow–up in a small case series by Gookin et al. (2010).

Koster et al. (2015) presented a retrospective case series also describing the use of 30 mg/kg ronidazole q 24 hours for 14 days in cats presented to a hospital environment with *T. foetus* confirmed via faecal smear microscopy and PCR. This dosing schedule was found to successfully resolve diarrhoea in 24/29 cats, however confirmation of resolution of infection via faecal PCR was not undertaken and there was no follow-up to determine whether relapse occurred.

The use of 30 mg/kg ronidazole q 24 hours for 14 days was also documented by Reinert et al. (2016) in a small cohort study and found to be successful in eradicating infection (demonstrated via faecal PCR) and preventing relapse up to 800 days following treatment completion. Presence of diarrhoea was not described. However, this was a small cohort study of five treated cats with no control or comparator group to substantiate the result.

A confounding factor for both the Koster et al. (2015) case series and Grellet et al. (2017) randomised, controlled study is that treated cats which continued to live in a multi-cat household environment were included. In both studies, a proportion of cats did not respond to treatment/remained infected, however it is impossible to know why treatment failure occurred as this could be a demonstration of resistance of *T. foetus* to ronidazole therapy, or simply reinfection.

Therefore, the use of 30 mg/kg ronidazole q 24 hours for 14 days may be a useful initial treatment regimen in eradicating infection with *T. foetus* and resolution of diarrhoea, with a successful result documented in the randomised, controlled study by Grellet et al. (2017) and supported by the cohort study by Reinert et al. (2016) and case series by Koster et al. (2015). However further case controlled, analytical studies should be undertaken to substantiate this. The case series and cohort studies are subject to bias and the result of the randomised, controlled study is complicated by the test and placebo cats not being housed separately during treatment, leading to possible reinfection.



Ronidazole appeared to be superior in efficacy against *T. foetus* compared to metronidazole and tinidazole (Gookin et al. 2006 and Gookin et al. 2010) and was effective in eradicating infection in cats previously treated unsuccessfully with fenbendazole in the small cohort study by Reinert et al. (2016). However, two cats did not respond to repeated ronidazole administration in the case series by Gookin et al. (2010). Resistance *in vivo* was suggested, and *in vitro* resistance was demonstrated in aerobic conditions in one cat, although further studies on larger numbers of cats are required to validate this finding.

Ronidazole is suggested to have a narrow therapeutic range and neurological side effects are documented to occur with treatment (Gookin et al., 2017), however these were infrequently evidenced in this knowledge summary, with only Reinert et al. (2016) observing effects in 2 cats which resolved on pausing treatment. Lim et al. (2012) specifically described the use of high dose 50 mg/kg ronidazole 12 hourly for 14 days. This was found to be successful in eradicating infection on faecal analysis, however the presence of any neurological side effects was unfortunately not discussed. Gookin et al. (2006) also administered doses up to 50 mg/kg twice daily and no adverse effects were seen.

Conclusions

There is a small amount of good quality evidence for the use of ronidazole to treat cats with diarrhoea as a result of *Tritrichomonas foetus* infection, and a range of therapeutic protocols have been described. A dose of 30–50 mg/kg administered once to twice daily for 14 days appears to be successful in eradicating infection and may successfully prevent relapse of infection for up to 800 days, although the evidence for this is limited. Neurological side effects may be observed in cats treated with doses of 30 mg/kg and above. Resistance of *T. foetus* to ronidazole has been suggested (Gookin et al., 2010), however additional work is needed to substantiate this further.

The small body of evidence currently available would suggest that the use of ronidazole is efficacious in treating *T. foetus* in cats and does resolve the associated diarrhoea, supporting the use of ronidazole in clinical practice in cats with diarrhoea and *T. foetus* confirmed via PCR/faecal culture. Based on this, the use of ronidazole as an off-license medication is therefore justified in the absence of a licensed alternative. The studies by Grellet et al. (2017), Koster et al. (2015) and Reinert et al. (2016) all suggest that a dosing schedule of 30 mg/kg once daily for 14 days is successful in treating infection, therefore may be a useful initial regimen. Based on the evidence generated by the search strategy for this knowledge summary, twice daily dosing and doses of up to 50 mg/kg may be required to eradicate infection and appeared to be safely tolerated. However, the studies detailing twice daily dosing are relatively old. A study by LeVine et al. (2011) investigating the pharmacokinetics of both intravenous and oral administration of ronidazole to cats observed a prolonged half-life of 10.5 hours for ronidazole.

Therefore, due to concerns about ronidazole accumulation and increased risk of neurological side effects occurring, twice daily dosing is no longer recommended (LeVine et al., 2011 and LeVine et al., 2014).

Importantly it appears that some cats may not respond to treatment and relapse may occur in a protracted period post-treatment, therefore patients should be monitored for this occurring.



Search Strategy	
Databases searched and dates covered:	CAB Abstracts via CAB Direct (1973–Week 8 2019) PubMed via NCBI website (1946–Week 8 2019) Scopus via Elsevier (1823–Week 8 2019)
Search terms:	(cat OR cats OR feline OR felis) AND Tritrichomonas(foetus OR fetus) AND ronidazole AND (efficacy OR susceptibility)
Dates searches performed:	24/02/2019–3/3/2019

Exclusion / Inclusion Criteria	
Exclusion:	Completely <i>in vitro</i> studies, review articles, studies not relevant to the PICO question, conference proceedings presenting overview or non-relevant information.
Inclusion:	<i>In vivo</i> studies, studies with more than one animal, studies relevant to PICO question, studies including treatment with ronidazole plus placebo and/or alternative antiprotozoal drug.

Search Outcome						
Database	Number of results	Excluded – Comment letter	Excluded – single case report	Excluded – irrelevant to PICO	Excluded – not accessible	Total relevant papers
CAB Abstracts	14	3	3	1	2	5
PubMed	10	2	1	3	0	4
Scopus	10	3	2	1	0	4
Total relevant papers when duplicates removed						6

CONFLICT OF INTEREST

The author declares no conflict of interest.



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Veterinary Evidence ISSN:2396-9776 **Vol 4, Issue 4** DOI: <u>10.18849/VE.V4I4.263</u> next review date: 03 Mar 2021



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