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Efficacy of a single dose of nitazoxanide in dogs naturally infected with *Giardia duodenalis*

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ABSTRACT

Giardia duodenalis is a protozoan parasite that infects many mammals, including dogs and cats. This waterborne and foodborne zoonosis is a major problem in one health. Treatment can be challenging because of long regimens and drug resistance. The objective of this study was to evaluate the efficacy of single-dose nitazoxanide (NTZ) for dogs naturally infected by *Giardia duodenalis*. Although widely used in humans, pharmacological safety is incipient, since the approval of the safe use of nitaxozanide for dogs is not a consensus in the world. Fifty dogs diagnosed with *G. duodenalis* by zinc sulfate flotation technique (Faust method) and cysts detection by light microscopy. Half of the animals received a dose of 50 mg/kg of NTZ and the other half received 3 doses of 50 mg/kg of fenbendazole (FBZ), both orally. One week after treatment, new fecal exams were done to prove the effectiveness. Of the animals treated with NTZ, 84% were negative for the protozoan, while 76% of the animals treated with FBZ were negative, no significant difference was identified. Side effects such as vomiting and hyporexia were manageable in NTZ treatment and no changes in laboratory tests showed hepatic or renal impairment. We conclude that the use of NTZ in a single dose of 50 mg/kg is effective for canine giardiasis, constituting an option to be considered for dogs with relapses, poor response to conventional drugs and to facilitate administration regimens.

1. Introduction

Giardia duodenalis (syn. *Giardia lamblia, Giardia intestinalis*) is a worldwide intestinal protozoan parasite that infects a wide range of hosts, including humans, domestic, and wild mammals (Moraes et al., 2019; Shakya et al., 2018). It proliferates in an extracellular and noninvasive fashion in the small intestine of vertebrate hosts, causing the diarrheal disease known as giardiasis (Moraes et al., 2019). The parasite can colonize the upper small intestine but it was also found in the lower small intestine, stomach, colon and biliary tract (Ballweber et al., 2010; Certad et al., 2017). For dogs, *Giardia duodenalis* infections can be asymptomatic, subclinical, and clinically evident forms may occur especially when other factors are also present, such as concurrent entero-pathogenic bacteria or parasites, food intolerance or decreased host defense ability and stress conditions. Recently, the prevalence of chronic diarrhea associated with *G. duodenalis* was 34% (Perrucci et al., 2020).

In the host, flagellated trophozoites adhere to the mucosa of the small intestine through the adhesion disc, but they constantly change location, damaging the intestinal mucosa. This infective form replicates by binary fission and eventually begins the encystation process to form cysts that will be eliminated in the environment (Ballweber et al., 2010; Certad et al., 2017). Cysts are then shed through host feces and were reported to remain viable for several months in water at temperatures below 10 °C and several weeks at room temperature. *Giardia duodenalis* is a zoonotic enteroparasite. Transmission of giardiasis occurs through the fecal-oral route, and may be either direct (i.e., person-to-person, animal-to-animal or zoonotic) or indirect (i.e., waterborne or foodborne) (Certad et al., 2017; Feng and Xiao, 2011; Moraes et al., 2019).

Giardiasis was formerly included in the WHO neglected diseases initiative and is directly associated with poverty and poor quality of drinking water (Savioli et al., 2006). *Giardia duodenalis* includes eight morphologically indistinguishable assemblages (A–H), of which assemblages C and D are found primarily in dogs, while *Giardia* assemblage A and B have zoonotic potential (Heyworth, 2016; Ryan and Zahedi, 2019). In Brazil, *Giardia* genotypes observed in dogs includes C and A assemblages (Figueiredo Pacheco et al., 2020; Trevisan et al., 2020).

From the above aspects, the treatment of animals positive for *Giardia*,

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symptomatic or asymptomatic, is a priority for pets as attention to one's health (Ryan and Caccio, 2013; Ryan and Zahedi, 2019). The treatment of giardiasis is a challenge for veterinarians and therapeutic failures are related to i) re-infection, ii) poor adherence (which involves cost, easy administration, low rate of side effects, among others), and iii) resistance to drugs. Several compounds have been tested against *Giardia* infections in dogs as benzimidazoles, in particular FBZ, metronidazole, ronidazole and secnidazole have activity against the parasite (Table 1) (Fiechter et al., 2012, Fenimore et al., 2017, Karahallİ and Ural, 2017, Faure et al., 2018, Cheung et al., 2019, Ciuca et al., 2021). Although with high efficacy, a FBZ resistance may occur in dogs (Barr et al., 1994; Da Silva et al., 2011; Emery-Corbin et al., 2021).

NTZ is a nitrothiazole antibiotic widely used in the treatment of human giardiasis, including children. The most accepted anti-infective mechanism of NTZ involves impairment of the energy metabolism in anaerobic pathogens by inhibition of the pyruvate: ferredoxin/ flavodoxin oxidoreductase (PFOR) (Pilla et al., 2020). Clinical study in shelter dogs with giardiasis showed better efficacy of NTZ at single dose of 75 mg/kg or 150 mg/kg, with repetition after 15 days, compared to the association of febantel with praziquantel and pyrantel for 3 consecutive days, but 37.5 mg/kg did not eliminate the infection (Moron-Soto et al., 2017).

Therefore, this study evaluated the efficacy of NTZ a single dose of 50 mg/kg in dogs naturally infected by *Giardia.* Additionally, the safety of the administered dose was evaluated, since the approval of the safe use of nitaxozanide for dogs is not a consensus in the world. In addition, single-dose treatments are most effective for aggressive dogs and for dog populations in kennels and shelters.

2. Materials and methods

2.1. Ethics statment

All procedures performed in this research were approved by the Ethics Committee for the Use of Animals of Universidade Paulista – UNIP (CEUA – protocol number 7056220419), in accordance with the national guidelines for ethics in research. Dog owners were informed about the study protocol and they gave their consent to inclusion of their pets. Those responsible for the animals signed an informed consent form.

2.2. Animals testing and study design

Fifty naturally *Giardia* infected dogs and with diarrhea were admitted to the study. Animals were allocated in groups randomly in order two groups of 25 animals each with similar parasitic loads of *Giardia* cysts:

- Fenbendazole Group - FBZ (25 animals) - all animals in this group, after diagnosis, were treated with FBZ, at the usual dose of 50 mg/kg every 24 h, for 3 consecutive days, with repetition after 15 days, as recommended.

- Nitazoxanide Group - NTZ (25 animals) - all animals in this group were treated with NTZ in a single oral dose of 50 mg/kg, with repetition after 15 days.

All animals (both groups) were positive for coproparasitological examinations (Faust method) of 3 fresh fecal samples obtained every other day from 7 days after the end of treatments (evaluation of the effectiveness of treatments from fecal analysis - presence of cysts of *Giardia duodenalis*).

2.3. Assessment criteria and clinical outcome

2.3.1. Inclusion criteria

The animals in this clinical study were domiciled dogs, at least 2 months old, without distinction as to gender, body score, breed or reproductive status (neutered and non-neutered), with a confirmed diagnosis of *Giardia*. For parasitological analysis, individual fresh fecal samples collected from each dog were examined within 24 h by Faust method (33% ZnSO4 solution, specific gravity 1.18). Approximately 2 g of feces are mixed with 15 ml of a 33% solution of zinc sulfate, strained, the tube filled with additional zinc sulfate, and centrifuged for 3–5 min at 1500 rpm. The liquid formed a convex dome (meniscus) which was collected with a platinum inoculation loop, transferred to a glass slide, stained with Lugol's solution (Synth®), covered with a coverslip, and screened under a light microscope (Nikkon®) under low power (100×) magnification and high power (400×) magnification. Positivity for *G. duodenalis* of this method was assumed to indicate the positivity of examined samples.

2.3.2. Exclusion criteria

Dogs affected by pre-existing diseases of a serious nature, such as liver diseases, nephropathies, endocrinopathies, malignant neoplasms were excluded from this study. Animals that use raw food were also not evaluated. All received only commercial diet (super premium food).

2.4. Follow-up of drug safety

Dog's owners were instructed to identify potential relevant events during the therapy, such as vomiting, inappetence, anorexia, apparent discomfort, excessive flatulence, noticeable nausea, prostration, excitement, change in urinary color, dry feces, among others. In addition, to having been oriented on remote access with the responsible researchers in case of doubts. Specifically regarding the need to establish

Table 1
Comparison between the drugs used in the treatment of giardiasis in dogs.

	Posology	Duration of treatment	Efficacy	Reference	Clinical side effects
Metronidazole (Nitroimidazole Family)	25 mg/kg twice a days	5 consecutive days 7 consecutive days	70.8% Plus *Enterococcus faecium* (SF68) and a standardized diet enhance clinical responses	Ciuca et al. (2021) Fenimore et al. (2017)	Neurotoxicosis with seizures and coma, have been reported in dogs receiving higher dosages or prolonged treatment Causes dysbiosis (Suchodolski, 2016; Rudinsky et al., 2022)
Secnidazole (Nitroimidazole Family)	30 mg/kg	single oral dose	90%	Cheung et al. (2019); Karahallİ and Ural (2017)	No adverse reactions Risk of dysbiosis unknown
Ronidazole (Nitroimidazole Family)	30 or 50 mg/kg, once a day	5 or 7 consecutive days		Fiechter et al. (2012)	Neurotoxicosis Risk of dysbiosis unknown
Fenbendazole (Benzimidazole Family)	50 mg/kg, once a day	5 consecutive days 3 consecutive days	80.9% 90%	Ciuca et al. (2021) Faure et al. (2018)	No side effects reported Minimal alterations for microbiome (Fujishiro et al., 2020)
Nitazoxanida	75 mg/kg, once a day	single oral dose		Moron-Soto et al. (2017)	Vomiting Risk of dysbiosis unknown

pharmacological safety before (baseline samples) and after the use of NTZ, biochemical tests were performed regarding serum urea and creatinine (renal profile analysis) and serum alkaline phosphatase - FA and alanine aminotransferase - ALT (liver profile analysis) before (5 to 7 days before receiving medication) and after (3 to 5 days after receiving medication).

3. Results

3.1. Distribution of positive cases by gender, age, breed and symptoms

Animals randomly included in NTZ group ($n = 25$) were 16 males and 9 females, ranging from 65 days to 14 years of age (Table 2). Among animals included in the FBZ group (n = 25), age ranged from 6 months to 12 years (Table 2), with 15 males and 10 females (Table 2). Together, the youngest animals (with 1 year of age or less) and young adults (between 1 and 6 years of age) constituted mostly of the study group (41/50). Regarding racial predisposition, there was a majority of animals (17) of mixed breed (SRD), followed by breeds: German Shepherd (4), Standard Poodle (4), German Spitz (4), Boston Terrier (3), Yorkshire Terrier (2), Golden Retriever (2), French Bulldog (2), and 1 Collie, Doberman Pinscher, Dalmatian, Welsh Corgi Cardigan, English Cocker Spaniel, Pug, Shih Tzu, Lhasa Apso, Border Collie and Rottweiler. As for the presence or absence of clinical manifestations, 40 animals were symptomatic for intestinal infection caused by *Giardia duodenalis* with diarrhea and 10 animals, although positive, did not exhibit clinical manifestations.

3.2. Treatment efficacy

After treatment, we observed that 84% (21/25) of animals treated with NTZ were negative for *Giardia duodenalis*, as well as clinical remission of symptomatic animals (Table 2). Only 4 animals (16%) had persistence of infection after treatment with clinical findings and positive tests for *Giardia* (of these 4 positive animals after treatment, 2 maintained clinical manifestations and 2 had remission of diarrhea). The efficacy of treatment with FBZ was 76% (19/25) and persistence of infection occurred in 24% of animals (6/25) (Table 2), showing that NTZ was as effective as conventional treatment with FBZ.

Among the animals that responded positively to treatment with NTZ (21 animals), 5 dogs had not shown an effective response to previous treatment with FBZ or secnidazole, a fact that is highlighted here because NTZ can be used as an alternative to conventional treatments. Among the 4 animals that did not respond to treatment with NTZ, several episodes of vomiting (2 or more) were observed immediately after taking the medication, which is hypothetically the cause of medication failure, since the drug may have been eliminated with the gastric contentes.

3.3. Side effects and laboratory findings

Among the side effects attributable to NTZ, there was an incidence of emesis in 45% of the animals (11/25), lethargy and transient hyporexia in 32% (8/25), nausea and mild drooling in 8% (2/25) and acute dermatological reaction with facial edema and urticaria in 4% (1/25), the latter alteration being noticed only in the youngest patient in this group (poodle dog, 65 days old). Such emetic episodes ceased within 1 to 3 days. Eleven animals (45%) did not exhibit any detectable clinical change. Two animals that showed significant emesis, did not continue the treatment and were out of the research and were duly rescued and guided by the researchers of this clinical study. No relevant changes were observed in renal (urea and creatinine) and hepatic (AF and ALT) functions (Suppl. Table 1). The animals in the FBZ group did not exhibit any side effects.

4. Discussion

A variety of drugs, such as benzimidazoles, furazolidone, 5-nitroimidazole compounds have been used in therapy against giardiasis. Unfortunately, single-drug and multidrug resistance have been

Table 2
Distribution of sample dogs according to treatment with nitazoxanide (NTZ) or fenbendazole (FBZ), age, gender and result of coproparasitological for *Giardia* after treatment.

		Nitazoxanide				Fenbendazole	
Dogs ID	Gender (Female/ Male)	Age (Months)	*Giardia* Positive/Negative after NTZ treatment	Dog ID	Gender (Female/ Male)	Age (Months)	*Giardia* Positive/Negative after FBZ treatment
N1	M	24	P	F1	F	48	P
N2	M	72	P	F2	F	36	N
N3	F	10	N	F3	M	12	N
N4	M	24	N	F4	M	144	N
N5	F	72	N	F5	M	60	N
N6	F	120	N	F6	M	6	P
N7	M	24	N	F7	M	144	P
N8	M	120	N	F8	M	12	N
N9	F	36	N	F9	M	12	N
N10	M	48	N	F10	M	24	N
N11	M	12	N	F11	F	60	N
N12	M	10	N	F12	F	8	N
N13	F	2	N	F13	F	7	N
N14	M	24	P	F14	M	11	N
N15	F	132	N	F15	F	48	P
N16	M	180	P	F16	M	3	N
N17	M	36	N	F17	F	8	P
N18	F	24	N	F18	M	72	N
N19	F	12	N	F19	M	132	N
N20	M	8	N	F20	F	96	P
N21	M	7	N	F21	F	108	N
N22	M	11	N	F22	M	12	N
N23	M	36	N	F23	M	12	N
N24	M	9	N	F24	M	24	N
N25	F	3	N	F25	M	72	N
			4 Positives and 21 Negatives 84% of Efficay				6 Positives and 19 Negatives 76% of Efficay

demonstrated or induced in vitro, and these drugs have relevant side effects (Argüello-García and Ortega-Pierres, 2021; Cernikova et al., 2018; Müller et al., 2007; Reyes-Vivas et al., 2014). Infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and spread resistant microorganisms to others. For this reason, expanding the number of drugs available for the effective and safe treatment of canine giardiasis is an important target in the pet clinic. Although NTZ is widely used in human giardiasis, with efficacy and safety in a single dose, its use in veterinary medicine based on clinical evidence is incipiente. Our study showed that a single doses of NTZ showed the same efficacy as FBZ (3 days of treatment). We demonstrated efficacy of NTZ against *Giardia duodenalis* in dogs with a lower single dose of NTZ (50 mg/kg) than others authors (3 consecutive doses of 75 mg/kg and 150 mg/kg) (Moron-Soto et al., 2017).

Benzimidazole is commonly used to treat giardiasis in dogs and cats. Studies evaluating the fecal microbiome of healthy dogs showed that short-term use of metronidazole (Pilla et al., 2020) can cause a dysbiosis in addition to greater potential for liver and neurological toxicity in cases of doses above 30 mg/kg per day. FBZ has been shown to be safe for the gut microbiota (Fujishiro et al., 2020). There are no studies, for now, analyzing the intestinal microbiome of animals treated with NTZ, being a medication that was used in the past for cryptosporidiosis in cats and that in this study was shown to be effective in the treatment of giardiasis in dogs. In this study, although FBZ and NTZ have shown the same efficacy against *Giardia duodenalis*, the use of NTZ is a therapeutic option for recurrent giardiasis. In addition, the single dose facilitates therapeutic regimens in kennels.

Lower doses of NTZ may be pursued in future studies that aim to maintain its therapeutic efficacy with possible minor side effects. The change in urinary color occurred in all animals in this group (25/25) as seen in the human population when subjected to this therapy (Moraes et al., 2019).

Regarding biomarkers of renal (urea and creatinine) and liver (AP and ALT) functions, no relevant changes were seen, suggesting safety in the use of NTZ (Supplementary Table 1). On the contrary, the use of secnidazole, as a drug used in a single dose in the treatment of *Giardia* spp., determined several biochemical alterations, such as in hepatic transaminases (ALT and AST) and in triglyceride levels (Shakya et al., 2018).

Animals under 1 year of age and young adults (between 1 and 6 years old) constituted the population that prevailed in our sample, in agreement with the literature data that indicate greater susceptibility of puppies and young adults, in relation to the elderly. Also according to previous work, giardiasis in males was higher than in females (Moraes et al., 2019; Moron-Soto et al., 2017; Pilla et al., 2020).

However, some dog breeds that are more predisposed to intestinal diseases and microbiome changes (dysbiosis) are less likely to have giardiasis (chronic colitis in brachycephalic patients, pancreatic insufficiency in German shepherd, lymphangiectasia in Yorkshire Terrier, among other particularities (Trevisan et al., 2020).

Considering that there is a limited number of drugs that have giardicidal activity, the use of NTZ is an interesting alternative for the therapy of dogs because it is administered in a single dose and can be an alternative for dogs that have not responded to other treatments (Moraes et al., 2019; Moron-Soto et al., 2017). Since multiple therapies are being validated for giardiasis insoluble to single drug therapy, NTZ in a single dose of 50 mg/kg can be listed for such associations, with manageable side effects. Future studies with lower doses of NTZ on consecutive days may cause less emesis. In addition, we can suggest that in view of this occurrence of emesis as a side effect of the drug, it should be considered using it after food intake or after 8 h of receiving oral antiemetic medication as a preventive.

Parasitism can be the cause of diarrhea, but dysbiosis can compromise the ability of the microbiome to contribute to the animal's immunity, making it more susceptible to opportunistic parasites and chronic infections. Diseases that can commonly compromise the microbiome, such as Inflammatory Bowel Disease (IBD) and Exocrine Pancreatic Insufficiency (EPI) should be investigated in these patients who are refractory to antiparasitic treatments (Suchodolski, 2016). In cases of multidrug-resistant giardiasis unresponsive to conventional treatments (FBZ, Metronidazole or Praziquantel associated with Febantel) or to more modern treatments (Secnidazole, Tinidazole and NTZ), it is expected to at least control the diarrhea, although the epidemiological aspects of giardiasis asymptomatic are a threat. There is no consensus, but it is believed that supplements and adjuvants that improve the quality of the microbiome can indirectly collaborate in the fight against a parasitic disease that is difficult to control, that is, at the moment there is evidence of vitamin D-3, vitamin B 12, fibers, glucosamines and symbiotics that can enrich the immunity of the microbiome, although this is not yet fully elucidated.

In conclusion, although NTZ has shown a higher percentage of efficacy against gidiasis in our study, when compared to the group treated with FBZ, and despite the absence of toxicity in the renal and liver biochemical tests performed in this study, undesirable adverse effects in almost 50% of animals treated with NTZ (mainly emesis and lethargy), suggest that this medication should not be adopted as a first choice in Giardia duodenalis infections. Future studies should better investigate other safer doses and the advantages of using NTZ in dogs with giardiasis.

Declaration of Competing Interest

The authors Felipe Saab Romano and Maria Anete Lallo declare no Conflict of interests in papers publication "Efficacy of a single dose of nitazoxanide in dogs naturally infected with *Giardia duodenalis*".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.04.022.

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