A Field Clinical Study to Confirm the Efficacy & Safety of a Metronidazole-based Oral Suspension in Dogs Naturally Infested by Giardiasis: Comparison to Fenbendazole.

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KEY WORDS: Metronidazole-based, Giardiasis, Dogs

ABSTRACT
Canine giardiasis is a common parasitic infection that can lead to severe diarrhea. It is considered as potentially transmissible to humans and its contaminant form (oocyst) is very resistant in the environment. Metronidazole or parasiticides are registered in Europe to treat this disease, and metronidazole-based treatment is associated with high success rates but also is reported as having narrow safety margins. So far no oral liquid form containing metronidazole is available for dogs even though it would allow a more precise dose of medicine and limit the risks of side effects.

This multicentric, randomised, blinded study was conducted to confirm the clinical efficacy and safety of a flavoured oral metronidazole suspension (ERADIA™ oral suspension – Virbac), compared to a fenbendazole suspension, on dogs naturally infested by Giardia.

A total of 193 dogs of any breed and gender were recruited from client households in France and Germany, and 131 cases were evaluated for efficacy analysis. The dogs were screened for giardiasis at first visit using a rapid Giardia test. Owners of dog displaying positive Giardia test were then asked to collect 3 fecal samples before the second visit (2 to 4 days later) when (D0) they were randomly treated with either metronidazole (25 mg/kg BW, twice daily for 5 days) or fenbendazole (50 mg/kg BW once daily for 3 days). Three other fecal samples were collected at days 5, 6, and 7, before the last visit on day 8. General examinations were performed during the visits and stool consistency scored based on the Purina scoring system (diarrhea was defined as a score ≥4). Giardia Immunofluorescence Assay (IFA) counts were done on each collected fecal sample at a central laboratory. Geometric means of the pre- and post-treatment samples were used to calculate the percent reduction in cyst excretion versus baseline, which was compared between groups us-
ing, originally, a non-inferiority approach. Non-inferiority of metronidazole suspension compared with fenbendazole suspension was achieved when the lower bound of the 95% CI of the difference between groups was greater than -10%. Additionally, the difference between products was considered statistically significant (p < 0.05) if the value 0 was not included in this confidence interval.

Based on geometric means, the percentage of reduction in cyst excretion was of 91.9% (n = 73) and 30.3% (n = 58) in the metronidazole and fenbendazole groups, respectively, at the end of the study. The difference [95% CI] between groups was of 61.7% [39.6; 77.7], showing the non-inferiority of the metronidazole suspension compared with fenbendazole suspension and a statistical difference between groups (p < 0.05). A statistical difference was also observed at each sample time (day 5 to day 7). The percentage of dogs cured from diarrhea after treatment was 84.0% and 67.9% in the metronidazole and fenbendazole groups, respectively (NS). The metronidazole suspension was considered as “highly palatable or palatable” more frequently than the fenbendazole suspension (53.8% vs 31.0 %, p < 0.01; Fisher’s test). The percentage of AEs remained low (19.8% dogs with metronidazole and 11.5% with fenbendazole) and did not request discontinuation of either treatment.

In conclusion, ERADIA™ oral suspension given as recommended was highly efficient to decrease cyst excretion and cure diarrhea in Giardia infected dogs and was well tolerated.

INTRODUCTION

Canine giardiasis is a protozoan infection due to Giardia that is transmitted to the dog or cat by ingestion of cysts shed by animals or humans. Giardia (Giardia duodenalis in particular) is considered the most common enteric parasite in dogs and cats, even in well cared pets, with a prevalence in Europe > 20%.1,2 Cysts are excreted in the environment via feces and can then be ingested via contaminated water, food, fomites, or through self-grooming. Excystation occurs in the duodenum, and the trophozoites can then attach to the intestinal epithelium and multiply by binary fission before encystation and excretion in feces.3

Giardiasis can remain subclinical, and when clinical signs occur they can range from abdominal discomfort to severe abdominal pain and diarrhea (due to intestinal malabsorption and hypersecretion).3 Giardiasis in dogs and cats is considered to have a zoonotic potential and similar symptoms are observed in humans.4 Therefore, hygienic measures must be taken when a pet is diagnosed with Giardia.

Metronidazole, a nitroimidazole antibacterial and antiparasitic, is the treatment of choice for this disease. Indeed Giardia, like a few other protozoa and obligate anaerobe bacteria, possesses the enzyme able to activate metronidazole.5 The activated molecule can then bind and damage DNA and induce death of the parasite. Since a selected number of parasites or bacteria possess this activating enzyme, the activity spectrum of metronidazole remains tight. Metronidazole is then of particular interest for diarrhea induced by Giardia, especially when there is a concurrent overgrowth of Clostridia.4

Nowadays, very few medicines containing metronidazole are available for dogs and none exist as a liquid formula. However, this type of presentation is convenient for all sizes of dogs and especially for the small ones, contrary to tablets, which sometimes have to be cut in small pieces. Oral suspensions allow to give a more precise dose of medicine and should limit inaccurate dosing: appearance of side effects (overdosing) and development of pathogen resistance (underdosing).

In this study, an oral suspension containing metronidazole (ERADIA™ oral suspension, Virbac) was tested in dogs naturally infested with Giardia, as assessed with a rapid test. The efficacy of the metronidazole oral suspension to reduce the number of cysts excreted and to cure diarrhea was evaluated and compared to the results obtained with
a fenbendazole oral suspension. This latter medicine is a parasiticide, commonly used to treat giardiasis in dogs. This multicentric, randomised, blinded study was conducted in France and Germany, and 131 cases were evaluated for efficacy.

**MATERIAL AND METHODS**

This study was multicentric, randomised, blinded by separation of functions, and was positively controlled.

This study was conducted in compliance with the guidelines on Good Clinical Practices (CVMP/VICH/595/98-Final), the guidelines on efficacy requirements for anthelmintic (CVMP/VICH/832/1999), the Directives instituting a community code regarding veterinary drugs, the European statistical guidelines (Guideline on statistical principles for Veterinary Clinical trials and Guideline on the choice of the non-inferiority margin) and the recommendations of WAAVP (“Guideline for the evaluation of drug efficacy against non-coccidial gastrointestinal protozoa in livestock and companion animals”).

No ethical approval was necessary for this trial and it was approved by local authorities (ANSES, German Federal Authorities).

**Animals**

Dogs of any breed and gender were recruited from client households of the veterinary clinics. A household presenting a maximum of three dogs could be selected. For efficacy and safety assessment, only one dog per household was included in the trial. A total of 193 dogs in 44 study sites (in France and Germany) were recruited in order to obtain sufficient evaluable cases for efficacy.

To be included in the study, client-owned dogs of at least 2 kg body weight (BW) must have had a Positive Giardia Snap™ Test (IDEXX) at inclusion. Exclusion criteria were:

- Major medical crisis and/or clinical signs consistent with the need of fluids therapy
- Hospitalisation, or anaesthesia/surgery
- Hepatic or neurologic disease
- Anthelmintic treatment including benzimidazole drugs (fenbendazole, albendazole, oxfendazole, febantel) or a combination of febantel-praziquantel-pyrantel product administered less than 7 days prior to first dosing (D0)
- Immunosuppressive treatment (corticoids, azathioprine, cyclosporin), anticoagulants, 5-Nitroimidazole-based antibiotics or quinacrin administered less than 15 days prior to first dosing (D0)
- Dogs coming from a pound or shelter
- Females known to be pregnant or lactating.

**Treatment**

Dogs received either 25 mg/kg BW twice daily (2mL/10kg morning and evening), for 5 consecutive days of metronidazole (ERADIA™ oral suspension - Virbac) or fenbendazole (PANACUR™ small animal 10% suspension – MSD animal health) orally at a dose of 50 mg/kg BW (1 mL per 2kg BW), once a day for 3 consecutive days. Products could be given in the mouth or on the food.

In order to avoid recontamination, hygienic measures were highly recommended for all dogs of the household and included cleaning/bathing the dog with a shampoo, disinfecting floors, and cleaning and drying dog’s accessories (toys, bowls, bedding, clothing, etc.).

None of the following products were allowed prior (7 to 15 days before D0) and during the course of the study: Anticoagulant, immunosuppressive/immunomodulatory treatment (corticoids, azathioprine, cyclosporin), anthelmintic treatment, including benzimidazole drugs (fenbendazole, albendazole, oxfendazole, febantel), or a combination of febantel-praziquantel-py rantel product, 5-Nitroimidazole-based antibiotics, Quinacrin.

**Design**

Dogs included in the study were observed for 10 to 13 days from the first visit. After confirmation of the giardiasis during this first visit (rapid test), the dog was presented
to the veterinary clinic on day 0 (visit 2, 2 to 4 days later), and day 8 ± 1 day (visit 3). Clinical examinations were then performed and BW recorded. Three dogs’ stool specimens were collected by the owner before treatment and after ending the treatment (on days 5 to 7). They were handed to the investigator and sent to a central laboratory for analysis (Giardia IFA method’). The stool consistency was also scored by the investigator according to owner’s description of the latest feces observed and the Nestle Purina fecal scoring system (Diarrhea = fecal score ≥ 4; Normal stool = fecal score ≤ 3) was applied.

IFA method: A case was considered for efficacy analyses when at least one of the three pre-treatment samples presented ≥ 750 G. duodenalis cysts per gram (CPG) of feces

Statistical Analysis
This study was originally designed as a non-inferiority study with a margin set to 10%.

The statistical analysis was performed using validated statistical programs (SAS 9.3).

The percent reduction in cyst excretion was calculated as follows:

The number of cysts at baseline and at the end of treatment were calculated for each dog as the geometric mean of the numbers of cysts in the three different samples before and after treatment.

Using these criteria, efficacy was assessed using 95% confidence intervals of difference between metronidazole and fenbendazole suspensions calculated with the bootstrap method. Non-inferiority of the metronidazole suspension compared with fenbendazole suspension was inferred if the lower bound of the 95% confidence interval (CI) of the difference between groups is greater than -10% at the end of the study. Additionally, the difference between products was considered statistically significant (p < 0.05) if the value 0 was not included in this confidence interval. The percent reduction in cyst excretion was also calculated at each day (day 5, 6 and 7).

Physical examination data and fecal scoring were analysed descriptively and non-parametric tests were used when appropriate.

All adverse events were recorded.

Results
Out of the 193 enrolled animals, 15 dogs were withdrawn early from the study and did not complete the follow-up period (seven dogs did not meet eligibility criteria, two dogs were withdrawn for major medical crisis and/or clinical signs requiring fluids therapy, hospitalisation, or anaesthesia/surgery, one owner withdrew his consent, one dog was lost to follow-up, four dogs were withdrawn for owner non-compliance), so that 178 were evaluated for safety and palatability. After removing dogs either presenting major deviations to the protocol or when giardiasis was not confirmed by IFA or when all pre-treatment samples presented less than 750 CPG before treatment, 131 dogs were evaluated for efficacy.

- Percent reduction of cyst excretion

The number of cysts per gram (CPG) in fecal samples at baseline, before treatment, ranged between 0 and 1 116 000. There was no statistical difference in CPG counts between groups before treatment. The percentage of reduction in cyst excretion before and after treatment was of 91.9% (n = 73) and of 30.3% (n = 58) for the group treated with metronidazole and fenbendazole oral suspensions, respectively.

The difference [95% CI] between groups concerning this parameter was of 61.7% [39.6; 77.7]. The lower limit of confidence interval was higher than -10%, showing the non-inferiority of metronidazole versus fenbendazole suspension and a statistical difference was even observed, in favor of the metronidazole-based suspension.

Figure 1 shows the evolution of cyst reduction (in % compared to baseline) after treatment (Day 5 to 7). The difference [95% CI] of cyst reduction (in %) between groups
was of 62.8 [40.3; 79.7], 60.0 [36.7; 78.0] and 55.5 [30.6; 75.6] on days 5, 6 and 7, respectively. The lower limit of confidence interval was higher than -10% and the difference between groups was significant at each sample time.

- Fecal Scores
The median (IQR) scores decreased on Day 8 compared to Day 0 (before treatment) in the group treated with metronidazole but remained stable in the group treated with fenbendazole [3 (2 – 4) to 2 (2 – 3) versus 3 (3 – 5) to 3 (2 – 4), at Day 0 and Day 8, respectively].

The percentage of dogs presenting diarrhea (fecal score ≥ 4) before treatment and cured from diarrhea after treatment was of 84.0% (n = 25) and 67.9% (n = 28), with metronidazole and fenbendazole suspensions, respectively (NS, Table 1).

- Palatability
The metronidazole suspension was considered as highly palatable or palatable for 49/91 (54%) dogs while the fenbendazole suspension was considered as such for 27/87 (31%) dogs, suggesting that palatability was better for the metronidazole suspension (p < 0.05, Fisher’s exact test). It is noteworthy though that, irrespective of the treatment group, in more than half of the cases the dogs took the products directly in the mouth.

- Adverse Events (AE)
A total of 28 dogs presented an AE: 18 dogs (19.8%) treated with metronidazole and 10 dogs (11.5%) treated with fenbendazole. One dog (1.1%) in the fenbendazole group presented 2 SAEs (severe diarrhea). Concerning the relationship to treatment, 2 AEs (reported in one dog treated with metronidazole) were considered as Probable (A) and 17 were considered as Possible (B): fifteen occurred in 9 dogs treated with metronidazole and two occurred in one dog treated with fenbendazole. The most common AE was “Digestive tract disorders” (vomiting and diarrhea), with 14 (15.4%) in the metronidazole group and 6 dogs (6.9%) in the fenbendazole group affected.

The percentage of AEs and SAEs remained low and did not result in discontinuation of either treatment. Overall, both products were well tolerated during the study.

**Table 1:** Number of dogs (%) with a score ≤ 3 (no diarrhea) and ≥ 4 (diarrhea) after treatment among the dogs who had diarrhea (score ≥ 4) before treatment. No statistical difference in the number of dogs cured from diarrhea (Fisher’s exact test).

<table>
<thead>
<tr>
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<th>Metronidazole suspension (n = 25)</th>
<th>Fenbendazole suspension (n = 28)</th>
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</thead>
<tbody>
<tr>
<td>Fecal score ≤ 3</td>
<td>21 (84.0%)</td>
<td>19 (67.9%)</td>
</tr>
<tr>
<td>Fecal score ≥ 4</td>
<td>4 (16.0%)</td>
<td>9 (32.1%)</td>
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**Figure 1:** percent reduction in the number of cysts excreted in feces after treatment with metronidazole (blue) or fenbendazole (green) suspensions. Number of animals evaluated (n) are indicated in brackets and italic. *: statistical difference between groups (p < 0.05).
CONCLUSION AND DISCUSSION

This multicentric, randomised, blinded and positively controlled study was performed to confirm the clinical efficacy and safety of ERADIA™ oral suspension (Virbac), a flavoured metronidazole suspension, after oral administration in dogs naturally infested by giardiasis, within normal conditions of use in the field, in comparison with a reference product containing fenbendazole.

This study showed that the metronidazole oral suspension was not inferior to the fenbendazole suspension to treat giardiasis in the field. Reduction of cyst excretion in feces at the end of treatment was, in fact, statistically higher in the group treated with metronidazole (92%) than in the group treated with fenbendazole (30%), showing a better efficacy of metronidazole compared to fenbendazole for this parameter. However, both treatments were similar in terms of efficiency to cure diarrhea (84% and 67.9% of dogs were cured after treatment).

These results are consistent with another study showing that metronidazole appeared to be more efficient than fenbendazole to treat giardiasis in dogs,8 while another one showed the efficacy of fenbendazole to control cyst elimination.8 The duration of treatment differed though, and it is possible that a longer treatment with fenbendazole (longer than recommended) would have given similar results than with metronidazole.

Other molecules (ronidazole, oxfenbendazole, nitazoxanide, etc.) have also shown efficacy for giardiasis treatment in dogs,9,10,11 but metronidazole and fenbendazole remain commonly used.6 The choice of the drug to be used can be made according to concomitant diseases or infections. Anthelmintic drugs like fenbendazole can be used when nematodes or cestodes are also present, while metronidazole should be used when there is an overgrowth of Clostridia or for its anti-inflammatory properties.3

In all studies evaluating the efficacy of drugs or combination of drugs to treat giardiasis, the importance of hygienic measures are highlighted. They include (as advised in this study): cleaning the dog with a shampoo focusing on peri-anal region, cleaning and disinfecting floors (employing quaternary ammonium compounds), cleaning and drying toys, food bowls, clothing and pet bed. These measures are important to avoid recontamination of the animal and the contamination of other animals and owners.

Tablets containing metronidazole for dogs can now be found in some countries. These tablets often have to be cut in pieces to adjust the dose to small pets. A liquid formulation is then often more suitable to give the right dosage, especially to small dogs. Indeed, dosage and compliance are important for antibiotics to limit the development of pathogen resistance.

On top of the efficacy, palatability is an important criteria to take into account for a better compliance. In this trial, it was found that the metronidazole suspension was considered palatable more often than the fenbendazole suspension. Although most dogs took the products directly into the mouth, it is also possible with liquid formulation to put it on the food, making it easier to give it to some reluctant dogs.

Some adverse events occurred during the study, but they were mainly non serious digestive tract disorders commonly associated with this type of treatment.10 None of these AEs led to discontinuation of treatment. Furthermore, it is important to note that most of the dogs received authorised concomitant treatments and when an AE occurred, it was not always possible to discriminate the origin of the AE.

Therefore, this study showed that, with a good tolerance of both treatment, the metronidazole oral suspension was more efficient than the fenbendazole oral suspension to reduce cyst excretion and as efficient to cure diarrhea. Compared to solid formulation of metronidazole, the liquid formulation and good palatability of ERADIA™ oral suspension can be an advantage when administered to small dogs or on the food.
CONFLICTS OF INTEREST
All authors are or were Virbac’s employee at the time of the study

REFERENCES
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