





**Anthelmintic Efficacy of Different Therapeutic Approaches in the Treatment of
Gastrointestinal Helminths in Dogs with Natural Infestations**

**Eficácia Anti-Helmíntica de Diferentes Formas Terapêuticas no Tratamento de Helmintos
Gastrintestinais em Cães com Infestações Naturais**

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Abstract

Gastrointestinal helminths are significant pathogenic agents in domestic animals, with many species exhibiting zoonotic potential, thus emphasizing their importance not only in Veterinary Medicine but also in public health. In this context, the search for effective and easily administrable anthelmintic agents has gained prominence, aiming to control these infections and promote both animal and human health. Accordingly, this study aimed to evaluate the anthelmintic efficacy of products containing

Pyrantel Pamoate and Praziquantel in Vermkill® Suspension (commercial product 1), Pyrantel Pamoate, Praziquantel, and Fenbendazole in Vermkill® Plus Tablets (commercial product 2), and Pyrantel Pamoate, Praziquantel, and Fenbendazole in Vermkill® Plus Suspension (commercial product 3) for the control of gastrointestinal helminths in naturally infected dogs. The experiments were conducted in both puppies and adult dogs, with coproparasitological monitoring to analyze the reduction in eggs per gram of feces (EPG). Vermkill® Suspension demonstrated efficacy in puppies, showing significant EPG reductions from the 7th day and residual protection for up to 14 days against *Ancylostoma* sp., and *Toxocara* spp. Vermkill® Plus Tablets and Vermkill® Plus Suspension demonstrated efficacy against *Ancylostoma* sp., *Toxocara* spp., and *Trichuris vulpis*, with residual effects lasting up to 28 days in adult dogs. Therefore, the products proved effective in the control of gastrointestinal helminths in dogs.

Keywords: Helminthiases. Fenbendazole. Pyrantel Pamoate. Praziquantel. Zoonoses.

Resumo

Os helmintos gastrintestinais são agentes patogênicos de grande relevância para os animais domésticos, com muitas espécies apresentando potencial zoonótico, o que lhes confere importância não apenas na Medicina Veterinária, mas também na saúde pública. Nesse contexto, a pesquisa por anti-helmínticos eficazes e de fácil administração tem ganhado destaque, visando o controle dessas infecções e a promoção da saúde animal e humana. Sendo assim, este estudo teve como objetivo avaliar a eficácia anti-helmíntica dos produtos compostos por Pamoato de Pirantel e Praziquantel no Vermkill® Suspensão (produto comercial 1), Pamoato de Pirantel, Praziquantel e Fenbendazole no Vermkill® Plus Comprimidos (produto comercial 2) e Pamoato de Pirantel, Praziquantel e Fenbendazole no Vermkill® Plus Suspensão (produto comercial 3) para controle de helmintos gastrintestinais em cães naturalmente infectados. Os experimentos foram realizados em cães filhotes e adultos, com acompanhamento coproparasitológico para análise da redução de ovos por grama de fezes (OPG). O Vermkill® Suspensão demonstrou eficácia em cães filhotes, apresentando reduções significativas no OPG a partir do 7º dia e proteção residual de até 14 dias contra *Ancylostoma* sp., *Toxocara* spp. O Vermkill® Plus Comprimidos e Vermkill® Plus Suspensão apresentaram eficácia contra *Ancylostoma* sp., *Toxocara* spp. e *Trichuris vulpis*, com efeito residual de até 28 dias em cães adultos. Portanto, os produtos demonstraram eficácia no controle de helmintos gastrintestinais em cães.

Palavras-chave: Helmintoses. Fenbendazol. Pamoato de Pirantel. Praziquantel. Zoonoses.

1 Introduction

Gastrointestinal helminths commonly infect dogs and cats worldwide. These infections may be subclinical or may cause significant disease and even death in severe cases (Becskei *et al.*, 2020). Domestic dogs can harbor a variety of intestinal parasite species, many of which have zoonotic potential, highlighting their relevance to public health (Rehbein *et al.*, 2016).

Endoparasitism causes significant morbidity and mortality in puppies and kittens, especially in environments that promote a higher likelihood of transmission, such as shelters, thereby maintaining a high prevalence of helminths in these populations (Miller, 2020). Among intestinal helminths, *Ancylostoma* spp., *Toxocara* spp., *Trichuris vulpis*, *Strongyloides stercoralis*, and *Dipylidium caninum* are the most prevalent in dogs and cats in Brazil (Dantas-Torres, 2020).

Parasitism by *Ancylostoma* spp. is characterized by local consequences such as irritation and ulceration of the intestinal mucosa, digestive disturbances, and systemic effects like anemia caused by the hematophagous habit of this nematode (Ré *et al.*, 2011). *Ancylostoma* spp. is also the causative agent of cutaneous larva migrans (CLM) in humans, commonly known as "creeping eruption" (Andrade Júnior; Araújo; Medeiros, 2015).

Nematodes of the genus *Toxocara* are the main ascarids that infect dogs and cats (Traversa, 2012). In dogs, infection by *T. canis* can lead to clinical signs such as stunted growth, diarrhea, dehydration, and abdominal distension (Guex; Mattos, 2020). In addition to its impact on animal health, *Toxocara* spp. is of public health concern, being the etiological agent of visceral larva migrans (VLM) and ocular larva migrans (OLM) syndromes in humans (Machado; Achkar, 2003).

Infections by *Trichuris vulpis* in adult dogs can range from subclinical to clinical manifestations characterized by gastrointestinal signs, although they are generally considered less pathogenic than those caused by hookworms and ascarids (Raza *et al.*, 2018). In puppies, the most common clinical signs include weight loss, reduced growth rate, and increased susceptibility to secondary infections (Traversa, 2011).

Several drug classes are available for the treatment and control of intestinal helminth infections, with the most commonly used being benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones (Traversa, 2012). To avoid limitations in the use of anthelmintics—especially in cases of mixed infections and due to the development of parasitic resistance—the combination of active ingredients has proven to be an effective strategy to broaden the spectrum of action and improve the control of helminth infections in animals (Moser; Schindler; Keiser, 2019).

In this context, the present study aimed to evaluate the anthelmintic efficacy of commercial products for the control of gastrointestinal helminths in naturally infected dogs. The products tested included pyrantel pamoate and praziquantel in Vermkill® Suspension (commercial product 1), and pyrantel pamoate, praziquantel, and fenbendazole in Vermkill® Plus Tablets and Vermkill® Plus Suspension, commercial products 2 and 3, respectively.

2 Material and Methods

The experiments were conducted at the facilities of the Animal Reception and Welfare Center “Adson Pereira de Almeida,” located in the city of Cruz das Almas, Bahia, Brazil, in partnership with Universidade Federal do Recôncavo Bahiano, with collaboration from Labovet Produtos Veterinários Ltda. and its team. The study was structured into three distinct experiments, according to the anthelmintics tested and their respective dosages. All procedures were conducted in accordance with ethical guidelines for animal research.

2.1 Experiment 1: Evaluation of Pyrantel Pamoate and Praziquantel in suspension

This experiment was submitted to the Animal Ethics Committee (CEUA) of Labovet Produtos Veterinários Ltda. and approved under protocol number PCEF – 008/22 prior to the start of the study. The objective was to evaluate the efficacy of Vermkill® Suspension, composed of Pyrantel Pamoate and Praziquantel, administered orally in a single dose.

Twenty naturally infected puppies were included based on the following inclusion criteria: age under 1 year, both sexes, and no antiparasitic treatment in the past 30 days. Exclusion criteria included animals with concomitant diseases or behavioral issues that could interfere with handling. The study followed a single-group design, with all animals receiving treatment, as recommended in MAPA Ordinance Draft No. 35/2020 (Brasil, 2020).

Dogs were kept in their original environments, fed a standard commercial diet, and given free access to water. Sunbathing followed the kennel routine. Dosages were calculated based on body weight, following the manufacturer's instructions (1.0 mL/kg of body weight). Fresh fecal samples, weighing between 5 and 10 grams, were manually collected from the animals' living area after spontaneous defecation, using examination gloves and sterile, properly labeled collection containers. Samples were stored in thermal boxes and sent to the laboratory for same-day analysis.

Pre- and post-treatment samples were analyzed quantitatively using the McMaster technique (Gordon and Whitlock, 1939) and qualitatively using the Willis technique (1921).

2.2 Experiment 2: Evaluation of Pyrantel Pamoate, Praziquantel, and Fenbendazole in tablets

This experiment was submitted to the Animal Ethics Committee (CEUA) of Labovet Produtos Veterinários Ltda. and approved under protocol number PCEF – 009/18 prior to the study commencement. It evaluated the efficacy of Vermkill® Plus Tablets, composed of Pyrantel Pamoate, Praziquantel, and Fenbendazole.

Sixteen naturally infected adult dogs were included, distributed into two homogeneous groups (Control and Treated), with eight animals each. The study followed the VICH GL 19 guidelines. Dogs were required to be older than six months, weigh more than 5 kg, be healthy, and have no prior treatments. Additionally, they had to be naturally infected by different intestinal parasites.

Selection was based on the average egg count per gram (EPG) in fecal samples collected on Days -3, -2, and -1. Treated dogs received a single oral dose of one tablet per 10 kg of body weight, adjusted as needed, according to the manufacturer's recommendations. The control group received placebo tablets containing all excipients except the active ingredients; administered at the same times and under identical conditions.

Fresh fecal samples (5–10 g) were collected directly from the animals' environment after spontaneous defecation and stored in sterile universal containers properly labeled. They were placed

in thermal boxes with ice and taken to the laboratory, where they were analyzed on the same day.

Sample collection occurred on Days -7, -3, -2, -1, +1, +7, +14, +21, and +28. Coproparasitological analyses included quantitative methods (McMaster technique, Gordon and Whitlock, 1939) and qualitative methods (Willis, 1921; Hoffman, Pons and Janer, 1934) to identify different helminth genera. Helminths were identified based on the morphometric analysis of eggs, following the criteria described by Monteiro (2017).

2.3 Experiment 3: Evaluation of Pyrantel Pamoate, Praziquantel, and Fenbendazole in suspension

This experiment was submitted to the Animal Ethics Committee (CEUA) of Labovet Produtos Veterinários Ltda. and approved under protocol number PCEF – 003/19 prior to the beginning of the study. The experiment replicated the procedures described in Experiment 2, using the formulation Vermkill® Plus Suspension, composed of Pyrantel Pamoate, Praziquantel, and Fenbendazole.

The same 16 dogs were selected based on identical inclusion and exclusion criteria, and allocation into Control and Treated groups was based on the average EPG. The drug was administered orally in a single dose of 1.0 mL/kg of body weight, while the control group received the placebo under identical conditions.

Fecal samples were collected on the same days and following the same protocol as in Experiment 2 and analyzed using both quantitative and qualitative techniques. All analyses were conducted on the same day as sample collection, ensuring sample integrity.

2.4 Experimental Design and Statistical Analysis

The study was conducted using a randomized and blinded design, with one team responsible for clinical procedures and another for laboratory analyses. To determine the efficacy percentage of Vermkill® Suspension, the following formula was used, according to the Draft of MAPA Ordinance No. 35/2020:

$$\text{Efficacy (\%)} = (\text{MHAT} - \text{MHPT}) / \text{MHAT} \times 100$$

Where: MHAT = Mean Helminth Count BeforeT; MHPT = Mean Helminth Count After Treatment.

To determine the efficacy of Vermkill® Plus Tablets and Vermkill® Plus Suspension, the formula described in VICH GL 19 was used:

$$\text{Efficacy (\%)} = (\text{MHAC} - \text{MHAT}) / \text{MHAC} \times 100$$

Where: MHAC = Mean Helminth Count in the Control Group; MHAT = Mean Helminth Count in the Treated Group.

The mean EPG (eggs per gram) of animals treated with Vermkill® Plus Tablets was analyzed and compared between groups using Analysis of Variance (ANOVA), followed by Tukey's test at a

5% significance level, using the BioEstat 5.0 software.

The mean EPG of animals treated with Vermkill® Plus Suspension was compared between experimental groups using the Mann–Whitney test. Statistical analysis was performed using MedCalc Statistical Software, version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; available at <https://www.medcalc.org>; 2016).

3 Results and Discussion

In samples analyzed during Experiment 1, screening parasitological exams revealed that all the animals evaluated were infected with helminths, with 100% (20/20) testing positive for *Ancylostoma* sp. and 95% (19/20) for *Toxocara* spp.

Among the dogs included in Experiment 2, a diversity of infections was observed, with a prevalence of *Ancylostoma* sp. (79.3%), *Toxocara* spp. (16.4%), and *Trichuris vulpis* (3.5%). In Experiment 3, in addition to *Ancylostoma* sp. (60%), *Trichuris vulpis* (24%), and *Toxocara* spp. (8%), oocysts of the protozoan *Cystoisospora canis* were also detected.

Consistent with these findings, studies such as those by Alves *et al.* (2016) and Ferraz *et al.* (2018), conducted in different regions of Brazil, show similar results, with *Ancylostoma* sp. being the most prevalent genus in dogs, followed by *Toxocara* spp. These results corroborate the findings of Ferreira *et al.* (2020), who evaluated the prevalence of gastrointestinal helminths in dogs seen at a university veterinary hospital in the state of Maranhão and identified these two parasites as the most common.

Similarly, in Rio Grande do Sul, Evaristo *et al.* (2018) conducted a study on the prevalence of gastrointestinal parasites in dog fecal samples collected from public squares in two cities. They found a prevalence of 96.77% for *Ancylostoma* sp. in Pedro Osório and 93.33% for the same genus in Cerrito. This high incidence of *Ancylostoma* sp. may be explained by the ability of L3 larvae to encyst in host muscle tissue for extended periods, becoming reactivated under stress and resuming their parasitic cycle, demonstrating the opportunistic behavior of this parasite (Castro *et al.*, 2019).

Regarding *T. vulpis*, Ferraz *et al.* (2020) identified this helminth as the second most prevalent species in a study conducted in Pelotas, RS, in which 1,356 fecal samples were analyzed, with 16% testing positive for *T. vulpis*. Similarly, Alves *et al.* (2016), in a study conducted in Pindamonhangaba, SP, reported *T. vulpis* as the third most prevalent helminth. These findings are in agreement with the results obtained in the present study.

Nijssen *et al.* (2014) identified significant differences in the prevalence of *Toxocara canis* among different age groups. Dogs aged 6 months to 1 year had the highest egg prevalence, followed by those

over 7 years and then those between 1 and 7 years. This supports the 95% prevalence of *Toxocara* spp. observed in fecal samples from animals under one year of age in Experiment 1, but contrasts with the lower prevalence found in Experiments 2 and 3 with adult dogs. This nematode is considered the main cause of helminthiasis in young animals (Macpherson, 2013), which supports these findings.

Following the administration of Vermkill® Suspension, coproparasitological analyses revealed a significant decline in EPG values among the treated animals (Table 1).

Table 1 – Mean \pm standard deviation of gastrointestinal nematode egg counts before and after treatment with Vermkill® Suspension

Nematodes	EPG Values Before Treatment	EPG Values After Treatment	p-value
<i>Ancylostoma</i> sp.	1847 \pm 1440 ^a	219 \pm 214 ^b	0.0001
<i>Toxocara</i> sp.	536.8 \pm 444 ^a	82.9 \pm 102 ^b	0.0001

(*) Means \pm Standard Deviation followed by different lowercase letters in the same row differ statistically from each other ($p < 0.05$).

Source: research data.

Regarding the anthelmintic treatment with Vermkill® Plus Tablets, the product demonstrated significant efficacy in reducing parasite egg counts in feces from the first day after treatment, maintaining significantly lower levels compared to the control group up to day 28 (Table 2).

Table 2 – Mean \pm Standard Deviation of gastrointestinal nematode egg counts in the control and treated groups (Vermkill® Plus Tablets) on different days after treatment

Day	EPG Values (Mean \pm Standard Deviation)		
	Control Group	Treated Group	p value
+1	3012.5 \pm 3818.2 ^a	25.0 \pm 46.3 ^b	0.0420
+7	875.0 \pm 517.5 ^a	112.5 \pm 318.2 ^b	0.0034
+14	1350.0 \pm 755.9 ^a	37.5 \pm 74.4 ^b	0.0004
+21	2162.5 \pm 2181.7 ^a	137.5 \pm 176.8 ^b	0.0194
+28	1607.1 \pm 943.6 ^a	385.7 \pm 203.5 ^b	0.0059

(*) Means \pm Standard Deviation followed by different lowercase letters in the same row differ statistically from each other ($p < 0.05$).

Source: research data.

Treatment with the anthelmintic Vermkill® Plus Suspension resulted in a significant reduction in helminth egg counts starting from the seventh day after treatment, maintaining significantly lower levels compared to the control group up to day 28 (Table 3).

Table 3 – Mean \pm Standard Deviation of gastrointestinal nematode egg counts in the control and treated groups (Vermkill® Plus Suspension) on different days after treatment

Day	EPG Values (Mean \pm Standard Deviation)		
	Control Group	Treated Group	p value
+1	2175 \pm 1388.5 ^a	2337.5 \pm 2775.9 ^a	0.8844
+7	712.5 \pm 533.0 ^a	187.5 \pm 383.4 ^b	0.0402
+14	2075.0 \pm 1636.9 ^a	375.0 \pm 942.3 ^b	0.0233
+21	1337.5 \pm 1135.1 ^a	25.0 \pm 46.3 ^b	0.0056
+28	1900.0 \pm 1660.5 ^a	162.5 \pm 220.0 ^b	0.0109

(*) Means \pm Standard Deviation followed by different lowercase letters in the same row differ statistically from each other (p < 0.05).

Source: research data.

In the efficacy analysis, it was observed that Commercial Product 1 (Vermkill® Suspension) demonstrated satisfactory results in all evaluations performed after its administration in puppies, showing efficacy from the 7th day and maintaining reduced egg counts through the 14th day (Table 4).

Table 4 – Efficacy percentage of the anthelmintic Vermkill® Suspension at different post-treatment intervals

Efficacy percentage (%)			
Treatment	Day +7	Day +10	Day+14
	92.8	91.9	97.8

Source: research data.

In the experiments with adult dogs, Commercial Product 2 (Vermkill® Plus Tablets) showed a significant reduction in egg counts starting from the 1st day post-treatment, with the best result observed on that day—99.2% efficacy (Table 5). The efficacy percentages remained satisfactorily high up to the 28th day after administration. In turn, Commercial Product 3 (Vermkill® Plus Suspension) caused a reduction in helminth egg counts starting from the 7th day post-treatment, maintaining this effect through the 28th day. The best result was recorded on the 21st day after administration, with 92.5% efficacy.

Table 5 – Comparison of the efficacy percentage of the anthelmintic Vermkill® Plus Tablets and Suspension at different post-treatment intervals

Treatment	Efficacy Percentage (%)				
	Dia +1	Dia +7	Dia +14	Dia +21	Dia +28
Vermkill® Plus Tablets	99.2	87.1	97.2	93.6	85.0
Vermkill® Plus Suspension	-7.4	73.7	81.9	85.0	92.5

Source: research data.

The anthelmintics, Commercial Product 2 and Commercial Product 3, are composed of a combination of the active ingredients: Pyrantel Pamoate, Praziquantel, and Fenbendazole. In contrast, Commercial Product 1 contains a combination of Pyrantel Pamoate and Praziquantel.

According to Bhanjadeo *et al.* (2022), various combinations of anthelmintic drugs are currently used to treat gastrointestinal parasitoses in dogs, aiming to increase treatment efficacy and broaden the spectrum of action.

Other authors have reported the efficacy of formulations using one or more of the active ingredients mentioned above, combined with other pharmacological classes. In a study conducted in Europe, Hayes, Wiseman, and Snyder (2021) evaluated the efficacy of Credelio Plus, a combination of Lotilaner and Milbemycin Oxime administered orally to treat gastrointestinal helminthiasis in naturally infected dogs. The results showed a 97.2% reduction in the mean egg count in fecal samples for *Toxocara canis*, *Ancylostoma caninum*, and *Trichuris vulpis*.

Becskei *et al.* (2020) evaluated the efficacy of Simparica Trio™, a chewable tablet containing Sarolaner, Moxidectin, and Pyrantel Pamoate. The study reported significant reductions in the mean fecal egg counts, reaching 99.0% efficacy against *Toxocara canis* in Europe and 99.2% in the United States. Rehbein *et al.* (2016) demonstrated the significant efficacy of a combination of afoxolaner and milbemycin oxime (NexGard Spectra®) against *Ancylostoma braziliense* (94.8%), *Ancylostoma caninum* (90.9%), *Toxocara canis* (97.8%), *Toxascaris leonina* (99.4%), and *Trichuris vulpis* (100%).

In Brazil, a study conducted by Risso *et al.* (2016) evaluated the efficacy of a combination of Praziquantel, Pyrantel Pamoate, Febantel, and Ivermectin in controlling *Toxocara canis* and *Ancylostoma caninum* in dogs housed at a university kennel. The animals received the treatment in tablet form, dosed at 3000 mg per 10 kg of body weight, with a repeated administration after 15 days. On the 30th day after the first treatment, coproparasitological tests showed 100% efficacy in controlling the parasites.

Oliveira *et al.* (2013) compared the efficacy of a combination of Fenbendazole, Pyrantel Pamoate, and Praziquantel with Mebendazole in treating dogs infected with *Ancylostoma* spp. in the municipality of Bom Jesus, PI. The results showed greater efficacy for the combination therapy

compared to Mebendazole, 15 days after treatment.

According to international guidelines for helminth control, established by the World Association for the Advancement of Veterinary Parasitology (WAAVP) and VICH GL19 (Veterinary International Cooperation on Harmonization), the minimum efficacy required for anthelmintic products is 90%. In contrast, the Brazilian Ministry of Agriculture and Livestock – mapa (Brasil) establishes a minimum efficacy of 80%.

In this study, efficacy was assessed based on the reduction of parasite burden, determined by egg counts in fecal samples. Treatment with Commercial Product 1 (Vermkill® Suspension) proved effective on days D+7, D+10, and D+14, with efficacy rates of 92.8%, 91.9%, and 97.8%, respectively. These results indicate that the product is suitable for treating puppies infected with gastrointestinal helminths, meeting international efficacy standards.

Commercial Product 2 (Vermkill® Plus Tablets) maintained high efficacy from the first day up to the 28th day post-treatment. Therefore, the anthelmintic is considered to have a satisfactory residual effect with prolonged action against gastrointestinal helminths in dogs.

Commercial Product 3 (Vermkill® Plus Suspension) showed its best efficacy on the 21st day and maintained this effect through the 28th day post-administration. This finding is particularly relevant, as it demonstrates the product residual effect and its potential to provide extended protection against helminths.

Both products maintained high efficacy levels between the third and fourth weeks post-treatment, covering the prepatent period of *Ancylostoma* sp. and *Toxocara* spp., which is 21 and 28 days, respectively (Monteiro, 2017). This characteristic reinforces the feasibility of single-dose treatments, reducing associated costs and minimizing the risk of selecting resistant parasites.

4 Conclusion

The results obtained allow us to conclude that Vermkill® Suspension, Vermkill Plus® Tablets, and Vermkill Plus® Suspension, administered orally in a single dose, were effective in controlling gastrointestinal helminths in dogs.

Vermkill® Suspension was effective in naturally infected puppies, providing residual protection for 14 days against *Ancylostoma* sp. and *Toxocara* spp.

Vermkill Plus® Tablets and Vermkill Plus® Suspension showed high efficacy against *Ancylostoma* sp., *Toxocara* spp., and *Trichuris vulpis* in adult dogs, with residual effects lasting up to 28 days.

These findings reinforce the effectiveness of the products in managing parasitic infections in

dogs, offering safe and long-lasting treatment options.

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