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Efficacy of pyrantel pamoate and ivermectin for the treatment of canine nematodes

Eficácia do pamoato de pirantel e da ivermectina no tratamento de nematódeos caninos

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Abstract

This study evaluated the efficacy of pyrantel pamoate and ivermectin on gastrointestinal nematodes in dogs. Fecal egg counts per gram (EPG) were measured by the fecal egg count reduction test (FECRT) in order to evaluate the anthelmintic efficiency and fecal float exams were also performed to assess the concordance between coproparasitological techniques. A total of 45 naturally infected dogs in the city of Bandeirantes, Paraná State, were selected and divided into three groups: Group 1, 15 animals that received pyrantel pamoate (145 mg) in a single dose; Group 2, 15 animals that received ivermectin (3 mg); and Group 3, 15 animals that comprised an untreated control group. Fecal testing was performed two and 10 days after treatment. *Toxocara* was the most prevalent genus, followed by *Ancylostoma* and *Trichuris*. *Ancylostoma* had low resistance to ivermectin and pyrantel pamoate treatment, while *Toxocara* were resistant to both treatments. Statistical correlation testing to compare coproparasitogical techniques revealed moderate concordance, substantial and almost perfect concordance for detection of *Ancylostoma*, *Trichuris,* and *Toxocara,* respectively. The results of this study suggest that the gender *Ancylostoma* had low resistance and *Toxocara* is resistant to both drugs and because of their high prevalence in young animals means that others anthelmintic drugs may be recommended to combat infections. Additionally, the Gordon and Whitlock modified and Willis-Mollay techniques are effective for detection particularly of *Toxocara* in dogs.
Key words: Ancylostomiasis, dogs, parasitic resistance, toxocariasis

Resumo

Objetivou-se neste estudo avaliar a eficácia do pamoato pirantel e da ivermectina sobre nematódeos gastrointestinais de cães. Para tanto foram realizados a contagem de ovos nas fezes (OPG) para avaliação da eficiência anti-helmíntica através do Teste de Redução da Contagem de Ovos nas Fezes (RCOF) e exame de flutuação Willis-Mollay para verificação de concordância entre as técnicas coproparasitológicas. Selecionou-se 45 cães na cidade de Bandeirantes, Paraná, naturalmente infectados, os quais foram divididos em três grupos experimentais: Grupo 1, 15 animais que receberam pamoato de pirantel 145 mg; Grupo 2, 15 animais que receberam ivermectina 3 mg e Grupo 3, 15 animais do grupo controle, não tratados. Coletas de fezes foram realizadas dois e dez dias depois para realização dos exames coproparasitológicos. Observou-se que o gênero de maior prevalência foi *Toxocara,* seguido de *Ancylostoma* e *Trichuris*. *Ancylostoma* apresentou baixa resistência ao tratamento

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com pamoato de pirantel e ivermectina, e *Toxocara* foi resistente a ambos os tratamentos. Através de testes de concordância estatística entre as técnicas coproparasitológicas utilizadas, foram constatadas concordâncias moderada, quase perfeita e substancial, respectivamente, na detecção de *Ancylostoma*, *Toxocara* e *Trichuris*. Concluímos neste estudo que o gênero *Ancylostoma* apresentou baixa resistência e *Toxocara* é resistente à ambas as drogas e, devido às suas altas frequências em animais jovens, devem ser indicadas outras drogas anti-helmínticas para o combate destas parasitoses. Adicionalmente, as Técnicas de Gordon e Whitlock modificada e Willis-Mollay podem ser utilizadas para detecção principalmente de *Toxocara* em cães.
Palavras-chave: Ancilostomíase, cães, resistência parasitária, toxocaríase

Introduction

The close proximity between humans and domesticated animals offers many social and emotional benefits for people of all ages. Among domesticated animals, dogs have a particularly close relationship with humans (PASQUA; PEDRASSINI, 2012). However, this relationship is less beneficial when dog health threatens human health (MACPHERSON, 2005).

Among infections common in dogs, gastrointestinal nematodes are of particular concern for veterinary and public health reasons (CASTRO et al., 2005). The most significant zoonotic intestinal nematodes are *Ancylostoma ssp, Toxocara canis* (LABRUNA et al., 2006), and *Trichuris vulpis* (LEITE et al., 2007). These parasites generally cause gastrointestinal upset, anemia, and weight loss in dogs, but severe cases may lead to death (PINTO et al., 2007). In humans, *A. caniunum* and *T. canis* parasite are responsible for cutaneous and visceral larva migrans, respectively (FIGUEIREDO et al., 2005), as well as gastrointestinal disturbances, anemia, and abdominal pain in *T. vulpis* infections (LEITE et al., 2007).

Gastroenteritis may occur in dogs of all ages, but is most common in neonates and animals less than six months of age, primarily due to their immature immune systems and transmammary and transplacental transmission (BOWMAN et al., 2010; RAMIREZ-BARRIOS et al., 2004).

Several drugs are commonly used to treat infected animals, including fenbendazol, mebendazol, pyrantel pamoate, febantel, praziquantel, milbemycin oxime, ivemectin, as well as similar combination drug therapies (HALL; SIMPSON, 2000; WILLIAMS, 1997). However, incorrect use these antiparasitics has resulted in increased parasite resistance against some chemical compounds (HOPKINS et al., 1998; KOPP et al., 2007).

Molento (2001) recommended administration of appropriate dosages for most effective use of specific chemistries; he also suggested continuous treatment for disease prevention, single-drug administration until proven parasite resistance, and use of anti-helminthic combination therapy only with previous knowledge about its efficacy (minimum 95%).

In this context, therefore, this study evaluated the efficiency of two anti-helminthic drugs pyrantel pamoate and ivermectin in dogs naturally infected with gastrointestinal nematodes, and assessed the concordance in results using Willis-Mollay and modified Gordon and Whitlock fecal examination techniques.

Material and Methods

A total of 45 dogs living in Bandeirantes city in Paraná state were included in this study. These dogs were less than one year of age, of both genders, and of different breeds. Animals were selected for this study based on their positivity for gastrointestinal nematode based on coproparasitologic exams using eggs counts per gram of feces (EPG) as determined by modified Gordon and Whitlock (1939) (1:12, 5 dilutions) and Willis-Mollay (WILLIS, 1921) techniques. The same researcher collected and analyzed all excrement samples within two days of

collection. The dogs were enrolled in this study and examinations were performed in spring and summer 2013 and 2014.

The animals were divided into three experimental groups: G1 consisted of 15 dogs that received 145 mg oral pyrantel pamoate (one pill per 10 kg weight) at day zero according to laboratory recommendations; G2 consisted of 15 dogs that each received 3 mg oral ivermectin (one pill per 10 kg weight) at day zero; and G3, the control group, consisted of 15 dogs that received no anti-helminthic drug treatment.

Coproparasitologic exams were repeated two days after drug administration to verify drug action and egg elimination as evidence of reduced helminth populations; fecal egg count reduction tests (FECRTs) were performed after 10 days. Samples from the G3 (control) group were re-examined only at day 10.

The average EPG was calculated for fecal samples for each group and each experimental day (zero, two, and 10); this value was used to calculate the FECRT, as described by Coles et al. (1992). The FECRT values were used to determine levels of anti-helminthic resistance using the following formula:

FECRT = 1 – (average EPG of the treated group on a specific day/average EPG from the same group at day zero) × 100.

Based on work by Coles et al. (1992), and classifications guidelines from the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.), helminth resistance was defined as FECRT results less than 95% 7 to 14 days after treatment. The current study also considered the Vizard and Wallace (1987) classification, which defined low resistance when FECRT test results higher than 95%, with a lower limit of the 95% confidence interval (CI) below 90%. A sensible classification when FECRT test must be higher than 95% and the trust interval must be higher than 90%.

EPG values (log x+1) from animals on the same group on different days and on the same day were compared using paired and unpaired t-tests, respectively. For all analysis, $p < 0.05$ were considered statistically significant. Data means and standard deviations were calculated and statistical tests performed using Graph Pad Prism version 5.0. The statistic concordance index (Kappa coefficient calculation) was calculated using EpiTable (EPI 6-CDC, Atlanta).

Results and Discussion

Among examined animals, 51.1% were male and 48.8% were female. Dogs of unknown breeding comprised 93.3% of cases and 73.3% were less than six months of age.

The most frequently identified genus was *Toxocara* (93.33%), followed by *Ancylostoma* (17.77%) and *Trichuris* (2.22%). These finding are similar to those reported by Fisher (2003), Labruna et al. (2006), Sager et al. (2006), Táparo et al. (2006), Funada et al. (2007), and Pedrassani and Zuco (2009), in fecal exams of dogs less than one year of age. *Toxocara* is the most common genus, found in 81% of dog populations (PRATES et al., 2009).

However, some studies have reported *Ancylostoma* to be more prevalent than *Toxocara* in dog excrement samples, including Kopp et al. (2007), who analyzed animal between three and six months of age; Lorenzini et al. (2007) who studied animals one year of age; and Prates et al. (2009), Ramirez-Barrios et al. (2004), and Swai et al. (2010), who analyzed dogs in different age ranges. Dogs of all ages can be infected by *A. caninum* because they do not develop effective immunity against this helminth (BLAZIUS et al., 2005; BOAG et al., 2003).

According to Labruna et al. (2006), *T. canis* infection in puppies less than six months of age may result fecal excretion of eggs released by adult parasites in the small intestine. Freitas (1977) and Barriga (1991) proposed that development of

adult larvae is rare in dogs older than six months of age, because most larvae tend to undergo somatic migration and develop into a latent state. The estrus phase in female dogs may reactivate these larvae and transmit them to fetuses and newborns through the placenta and milk (BOWMAN et al., 2010). This transmission cycle may explain the high incidence of *Toxocara* in this study, since the majority of dogs (73.3%) were less than six months old.

The FECRT results (Table 1) revealed great reduction ($p < 0.05$) in nematode EPG after 10 days of treatment in both experimental groups. Significantly fewer nematode eggs were observed after two days in the group that had received ivermectin compared to the group that had received pyrantel pamoate ($p = 0.036$). The observed reduction of EPG during coming days in animals of different groups was due genre *Toxocara* probably because it happen more frequently in animal of the present study.

Another important observation in this study was the gradual reduction of EPG in fecal samples from dogs treated with pyrantel pamoate compared to the fast EPG decline and persistence in dogs treated dogs with ivermectin (Figure 1).

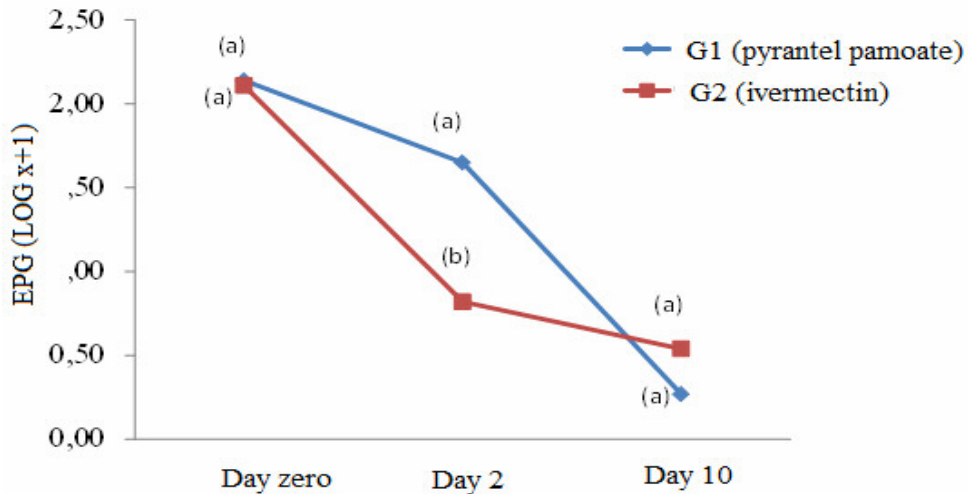
The FECRT results two and 10 days after pyrantel pamoate and ivermectin treatment are shown in Table 2.

Table 1. Egg counts per gram of feces (EPG) transformed in log (x=1) of experimental groups G1 (pyrantel pamoate) and G2 (ivermectin) before treatment, two and 10 days after treatment, and untreated control (G3).

	EPG Nematodes (LOG X+1)			EPG *Ancylostoma* (LOG X+1)			EPG *Toxocara* (LOG X+1)		
	Day zero	Day 2	Day 10	Day zero	Day 2	Day 10	Day zero	Day 2	Day 10
G1	2.46[(a)]	1.80[(a)]	0.39[(a)]	0.52[(a)]	0.16[(a)]	0.12[(a)]	2.14[(a)]	1.65[(a)]	0.27[(a)]
G2	2.28[(a)]	0.90[(b)]	0.62[(a)]	0.50[(a)]	0.24[(a)]	0.08[(a)]	2.11[(a)]	0.82[(a)]	0.54[(a)]
G3	2.54[(a)]	NA*	2.63[(b)]	0.12[(a)]	NA	0.08[(a)]	2.54[(a)]	NA	2.52[(b)]

Different letters in the same column represent significant differences ($p < 0.05$) between groups on the same day, as calculated using unpaired *t*-tests. *NA: Not accomplished.

Figure 1. Changes in egg counts per gram of feces (EPG log x + 1) before, two, and 10 days after treatment.



Different letters represent significant differences ($p<0.05$) between groups on the same day, as calculated using unpaired t-tests.

Table 2. Reduction in fecal egg count rate (% FECRT) of *Ancylostoma, Toxocara,* and *Trichuris* in the feces of dogs treated with pyrantel pamoate (G1) and ivermectin (G2) two and 10 days after treatment.

	FECRT (%) G1		**FECRT (%) G2**	
	Day 2	**Day 10**	DayaomplishedWhitlock (Schedule 4rmectine) 2	**Day 10**
Ancylostoma	94.2	98.3	85.7	95.2
Toxocara	56.8	93.5	68.9	93.5
Trichuris	NA*	NA	100	100

*NA: Not accomplished.

Ancylostoma EPG decreased by 98.3% two and 10 days after treatment in the group that received pyrantel pamoate. However, according to Vizard and Wallace (1987) classification, the IC was less than 90% (85%), suggesting low resistance to the drug. Carvalho and Araújo (2009) administered 15 mg/kg of the drug and reported a 100% reduction of *A. caninum* eggs seven days after treatment, better results than those observed in the current study.

However, Kopp et al. (2007) tested the efficacy of a 14.4 mg/kg dose of pyrantel pamoate in dogs artificially infected with *A. caninum*, reporting increased egg production and only 25.7% reduction of adult parasites in the intestines after six days of treatment compared with the untreated control group, suggesting parasite resistance to the drug.

Toxocara in this study appeared slightly more resistant to pyrantel pamoate, with an FECRT 10 days after treatment of 93.5%. This result is similar to that reported by Carvalho and Araújo (2009), who administered a single 15 mg/kg dose of the same drug to dogs infected with *T. canis,* resulting in an 80.73% egg reduction after seven days of treatment. In São Paulo state, Castagnolli et al. (1999) also reported a lower reduction (83.85%) after administration of 5 mg/kg of anti-helminthic to the same dogs.

Pyrantel pamoate belongs to the tetraidropirimidine family of drugs. Its mechanism of action is as a neuromuscular blocking agent, which causes parasite immobilization and expulsion from the host (REINEMEYER; COURTNEY, 2001). Gennari et al. (1997) reported that double or quadruple the supplier-recommended pyrantel pamoate dose was required for 100% *Toxoraca sp* egg reduction in cats. Moraes et al. (2004) reported that the manufacturer-recommended dose was not sufficient to eliminate 100% of adult roundworms.

In the present study, the *Ancylostoma* obtained 95, 20% of FECRT and the 79,1% of Confidence interval (CI). These findings indicate a low resistance to ivermectin. These results are different from those reported by Pimpão et al. (2005), who observed good efficacy against gastrointestinal nematodes in dogs who received 600 µg/kg intradermally. Bowman et al. (2010) also reported high efficacy of this drug at a dosage of 0.2 mg/kg.

Toxacara was resistant to this compound, with an FECRT of 93.9%. Lescano et al. (2005) tested this drug in rats artificially infected with *T. canis,* observing a significant reduction in parasite load, but no parasitological cure. However, other authors have reported a dose of 0.2 mg/kg to be effective against this helminth (BOWMAN et al., 2010).

Gerrero et al. (2002) reported that ivermectin (6 µg /kg) combined with pyrantel pamoate (5 mg/kg) was effective against *Ancylostoma, Toxocara, Trichuris,* and *Dirofilaria immitis* (heartworm). Nolan et al. (1992) also reported high efficiency against *A. caninum* (99.6%) using this formulation. These data suggest that these drugs appear to have a synergistic effect when used in combination. *Trichuris* were sensitive to ivermectin, but it was not possible to evaluate the efficacy of pyrantel pamoate in this genre because it was not identified in the day zero G1 samples in this study.

Tracking reduction in egg counts allows estimation of the anti-helminthic efficiency of drugs (VIZARD; WALLACE, 1987). We cannot forget that these parasites have zoonotic potential and that dogs in intimate in contact with humans may be important sources of infection (BOWMAN et al., 2010; LABRUNA et al., 2006).

The results of this study revealed low *Ancylostoma* and *Toxocara* resistance to pyrantel pamoate and ivermectin based on FECRT (93.5% and 98.3%, respectively). However, *Toxocara* is of particular zoonotic and pathogenic concern as the causative agent of visceral larva migrans in humans, especially children; veterinarians and owners would prefer reduction indexes closer to 100% to prevent disease spread. In this study, 20% of dogs treated with pyrantel pamoate as recommended by the supplier still shed *Ancylostoma* and/or *Toxocara* eggs up to 10 days after treatment. This proportion was larger in dogs treated with ivermectin, where 33.3% were still shedding eggs from both parasites.

Coproparasitologic examination performed using Willis-Mollay as well as modified Gordon and Whitlock techniques revealed Toxocara, Ancylostoma, and Trichuris prevalence of 63,3%, 11,7%, 3,33%, and 65,8%, 12,5%, and 1,67%, respectively (Table 3). However, Amarante et al. (2006) reported observing that the Willis-Mollay technique presented a higher sensibility for the detection of Ancylostoma and Toxocara eggs than the Gordon and Whitlock technique. In a similar study, Hijano et al. (2014) was also observed a higher sensitivity in the detection of eggs like in the Willis-Mollay technique. In this study, we credit the best sensitivity result of the Gordon and Whitlock technique at a 1 : 12,5 dilution, rather than 1:50 . This decreased excrement dilution may be useful for increased sensitivity.

Analysis of the statistical concordance between the techniques used in this study resulted in nearly perfect agreement ($k = 0.86$ $x^2 = 87.85$) for detection of *Toxocara*. Among other nematodes observed this study, substantial and moderate agreements were observed for *Trichuris* and *Ancylostoma* (Table 3) based on Landis and Koch (1977) criteria.

Table 3. Stool samples positive and negative for *Ancylostoma, Toxocara*, and *Trichuris* according to examination technique (Gordon & Whitlock vs. Willis-Mollay), and compliance statistics index (Kappa) between techniques.

	Willis-Mollay								
	Ancylostoma			***Toxocara***			***Trichuris***		
	Pos	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**
Gordon & Whitlock									
Positivos	9	6	15	73	5	78	2	0	2
Negativos	5	100	105	3	39	42	2	116	118
Total	**14**	**106**	**120**	**76**	**44**	**120**	**4**	**116**	**120**
Kappa (k)		0,57			0,86			0,66	
Valor de p		<0,0001			<0,0001			<0,0001	

Conclusion

The results of this study indicate that *Ancylostoma* have low resistance to pyrantel pamoate and ivermectin, while and *Toxocara* is resistant to both drugs, suggesting that isolated administration of those medicines is not sufficient for complete eradication. Furthermore, the Gordon and Whitlock modified technique showed similar efficacy to Willis-Mollay technique for detection of *Ancylostoma* and *Toxocara,* for studies of parasite frequency and anti-helminthic efficacy.

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