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Original Research Article

Clinical Evaluation of a New Generic Product with Milbemycin Oxime and Praziquantel on Dogs Naturally Infested with Toxocara canis and Dypilidium caninum

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Abstract

Although there are currently generic products for more than half of the veterinary dewormers in use, anthelmintics with Milbemycin oxime and Praziquantel are still insufficiently represented and promoted. The purpose of this study, which consists of the clinical and coproparasitological evaluation of the efficiency and risks of the generic product Milbenin in canine anthelmintic therapy, falls within this context. The research was carried out on 20 mixed-breed dogs (common breed; 7 males and 13 females), aged between 1-10 years, from a shelter administered by a private company for the protection and adoption of community dogs, from northwestern Romania. The study consisted of routine clinical examinations and the collection of individual faecal samples, followed by copro-parasitological investigations (n=20) using qualitative methods (modified Willis ovoscopic and Baermann larvoscopic) to evaluate the intensity of natural parasitism with *Toxocara canis* and *Dipylidium caninum*. Infested dogs were treated with Milbenin, one tablet for 5-25 kg of body weight, according to manufacturer's instruction. The results obtained revealed a very good therapeutic efficacy of this new generic product, expressed by the clinical recovery and coproparasitological negativity of 18 of the dogs, representing 90% of the sample of treated patients. According to the administered dose, the active substances achieved mean plasma concentrations of 1 (0.5-2.4) mg/kg for Milbemycin oxime and 10.9 (5-23.6) mg/kg for Praziquantel, respectively. Given the high prevalence of canine helminth infestations especially among the shelter dogs, the results of this study are relevant and timely for outlining the prophylactic-curative conduct of endoparasitism with a new generic product based on milbemycin oxime and praziquantel - highly active molecules with a broad antiparasitic spectrum.

Keywords: Dog, Dypilidiasis, Generic product, Milbemycin oxime, Praziquantel, Tablets, Toxocariasis

INTRODUCTION

The evaluation of the therapeutic efficacy and safety of pharmaceutical products also requires preliminary or additional clinical tests, carried out on patients from the target species. Currently, more than half of the medicines for human use can be replaced by one or more generic products, a situation that has also extended to veterinary drugs. Statistical differences between generic and original products are not allowed because bioequivalence

automatically implies the interchangeability of the tested products (Tolomeiu et al., 2020; Arion et al., 2018).

This study, which is intended to evaluate the therapeutic efficacy of Milbenin, a new generic product, in the form of tablets with 12.5 mg milbemycin oxime and 125 mg praziquantel, also falls within the presented context. One of the most common causes of gastrointestinal disorders is infestation with various helminths, which can cause significant losses in production farms due to morbidity and mortality in livestock. *Toxocara Canis* and *Dipylidium caninum* are among the gastrointestinal helminths (*Ancylostoma caninum, Giardia duodenalis, Capillaria aerophila, Strongyloides stercoralis*) that parasitize animals and can frequently cause zoonotic infestations, especially in immunodeficinet individuals, those with other associated pathologies, or in children (Mircean et al., 2017).

According to Schwartz et al., (2022), tens of millions of people and over one hundred million dogs are infected annually following the spread of *Toxocara canis* eggs in the environment. The prevalence of toxocariasis in Europe is 32%, ranking it among the most common canine gastrointestinal parasites (Schimmel et al., 2011). In Romania, due to the high rate of parasitic infections, *T. canis* has the highest prevalence among canine parasitic infestations. A coproparasitological study on 155 dogs from Cluj County showed a prevalence of 67.1%. The highest infestation rate was observed among puppies and in dogs that had not undergone proper deworming protocols (Ursache et al., 2017). Infested puppies may experience moderate to severe infections, leading to stunted growth, gastrointestinal symptoms such as diarrhea and abdominal discomfort, and, in severe cases, death due to mechanical obstructions like intus-susception, perforation, or intestinal obstruction (McTier et al., 2000).

A study conducted by Hoffman et al. found a *T. canis* infestation rate of 73% in a sample of 100 dogs from Gorj and Vâlcea counties, Romania. This helminth pollutes the environment with highly resistant invasive elements, which maintains the danger of direct transmission of the infection. Further research is needed to explore new active compounds, different concentrations, and novel formulations that can inhibit embryogenesis or completely inactivate *T. canis* eggs (Ursache et al., 2020). Complete eradication of *T. canis* is nearly impossible due to its multiple transmission routes and complex biological cycle (Hoffman et al., 2022). According to the European Scientific Counsel Companion, at least four deworming treatments per year are recommended for pet dogs, while in high-risk zoonotic areas, deworming should be performed 10-12 times per year (Nijsse et al., 2015). Milbemycin oxime has been used for over two decades to treat and control intestinal nematodes and dirofilariasis in dogs. A minimum dose of 0.75 mg/kg (Credelio Plus) has been proven effective against *T. canis* L4 larvae, *Angiostrongylus vasorum*, and immature adult stages of *A. caninum* (Young et al., 2021). Dipylidiasis, caused by *Dipylidium caninum*, is one of the most common canine parasitic infestations. It is often asymptomatic but can sometimes present with mild, nonspecific clinical signs. Its biological cycle is complex, requiring an intermediate host (lice or fleas), which is then ingested by the final host (carnivores or humans) to complete the parasitic cycle (Rousseau et al., 2022).

For the design of the current study, the knowledge and practical skills of veterinarians, statisticians and volunteers were used, which formed the basis for the implementation of a relevant protocol for the detection, control and prophylaxis of toxocariasis and heartworm disease, some of the most common canine parasitic diseases.

In the context of the above, the evaluation of a veterinary pharmaceutical product for its introduction on the market can be based on therapeutic equivalence testing or bioequivalence testing, thus comparing its relative bioavailability with that of a reference product (Ognean et al., 2012). Of the two procedures, the bioequivalence test is more accessible, because the latter one requires longer administration of the medicinal product and a large number of animals, which can multiply side effects (Crivineanu, M., Nicorescu, V., 2012). Under these circumstances, the motivation for carrying out the current study can be summarized as the need to estimate the prevalence and epidemiological impact in the control of toxocariasis and dirofilariasis as natural infestations in dogs maintained in shelters administered by local authorities of some localities in Hunedoara County, Romania. The aim of this study, focused on clinical, hematological and copro-parasitological investigations, is to evaluate the prophylactic-therapeutic efficacy and tolerance of a new generic product with Milbemycin oxime and Praziquantel, in natural infestations with *Toxocara canis* and *Dypilidium caninum*. This dewormer has been recently approved through bioequivalence testing on dogs as the target species and proposed for therapeutic interchange in canine helminthiasis.

MATERIALS AND METHODS

The current research intends to evaluate the therapeutic efficacy, adverse effects and tolerance of this new generic product, with a view to promoting it in canine anthelmintic therapy, and it followed the characteristic steps for this type of study.

Location of the Study

The research was conducted between September and December 2024, in a private community dog shelter of the Lupy organization in western Romania (Orăștie,

Table 1. Summary of individual data and descriptive statistics of the investigated batch

Subject /parameters	Age (years)	Weight (kg)
Females: 13 Males: 7		
Mean	3.5	14.65
Standard deviation	3.283	6.37
Range	0.5-10	5.3-25
Minimum	0.5	5.3
Maximum	10	25
Count	20	20

Table 2. General outline of the study and chronology of the investigations carried out

Authorized company for marketing	**Pharma VIM Korlátolt Felelősségű Társaság, Budapesta, Ungaria**
Series manufacturer	Vim Spectrum SRL, România
Generic products	Milbenin 12,5 mg/125 mg chewable tablets for dogs
Active ingredients	Milbemycin oxime, Praziquantel
Study design	Cohort studies
Experimental batch size	Minimum 20 dogs
Main selection criteria	Mixed breeds, male and female with a body weight of 5 and 25 kg
Dosage	One tablet (Milbemycin oxime 12,5 mg, praziquantel 125 mg)
Drug administration route	Oral
Length of treatment	Single dose
Study procedure (dosage)	Administration of a single tablet/animal
Clinical exam	Monitoring the temperature (^{0}C), pulse (no./min), respiration rate (no./min)
Coproparasitological exam	ovoscopic (modified Willis method), larvoscopic (Vaida method), reduction of parasitism intensity [initial score-final score/initial score x 100]
Hematological evaluation	Wet Mount test (blood smear)
Statistical analysis	Chi-square, prevalence
Monitoring of adverse reactions and assessment of tolerance	Describing the detected side effects (adverse reactions) and classification into the appropriate drug tolerance level (very good, good, satisfactory)
Evaluation of therapeutic efficacy	Post-therapeutic evaluation of the proportion of coproparasitological negativity

Hunedoara County).

Animals

All dogs were intended for adoption and they were maintained in proper conditions offered by this shelter. As it can be seen from the summary of descriptive statistical data of the investigated group of dogs, presented in Table 1, the organization of the experimental batch respected the minimal inclusion/exclusion criteria in the study. The research began with the weighing of each animal to calculate the appropriate dose before administering the product. Thus, only dogs with a weight between the minimum value of 5 kg and the maximum of 25 kg, males and non-pregnant/non-lactating females, which had not been dewormed in the last 3 months, and which tested positive for *Toxocara canis* and *Dypilidium caninum* during the preliminary coproparasitological examination, were included in the study.

The selection of subjects was based on the correlation of the results obtained during the medical history assessment, the general clinical examination and the preliminary coproparasitological testing of each animal. For this purpose, the basic physiological parameters were evaluated: body temperature, pulse (from the femoral artery), mucous membrane color, capillary refill time, heart and respiratory rate and the absence or presence of sensitivity following abdominal palpation. At the same time, a complex epidemiological investigation was carried out to establish the medical history, mainly recording the status of deworming and vaccinations. The preliminary coproparasitological examination followed. Simultaneously with the administration of the product, an inspection of the oral cavity was also performed. Throughout the study, the dogs were fed a standardized dry diet containing psyllium extract to enhance digestibility (Carnilove Adult Lamb and Venison).

Study Design

The general outline of the study and the chronology of the investigations performed are shown in Table 2. A preliminary evaluation of the animals was needed in order to organize the experimental batch composed of 20 dogs that met the inclusion criteria previously specified. On Day 0, the dogs were selected based on the inclusion criteria. Each dog was housed in an individual kennel for proper identification and fecal sample collection. On Day 1, the dogs underwent a 12-hour fasting period before and after tablet administration, with water available at all times. Clinical examinations were performed, and blood samples were taken to screen for microfilariae by using the Wet Mount test. A 3 mL venous blood sample was collected, on EDTA (by jugular or cephalic brachial vein), from each dog. Coproparasitological samples were collected on Days 1, 7, and 14 and stored at cold temperatures before examination to identify parasite species. The coproparasitological examinations were performed on the day of collection.

The evaluated anthelmintic product

The new generic product Milbenin 12.5 mg/125 mg chewable tablets for dogs was introduced into the study (Todoran et al., 2025). This product, with milbemycin oxime (12.5 mg) and praziquantel (125 mg), was formulated and approved based on the results obtained in bioequivalence testing by the company authorized to market it, SC Vim Spectrum SRL. Tablets from a series produced by this pharmaceutical company were introduced into the study (Table 2).

Coproparasitological Examinations

Fecal samples were collected individually, labeled, and stored at 4°C until analysis, which was performed within 1-4 hours of collection. The usual copro-parasitological examinations were performed to confirm infestations with *Toxocara canis* and *Dypilidium caninum* and to evaluate its intensity, resorting to ovoscopic (modified Willis method) and larvoscopic (Baermann method) testing. All fecal samples were examined using the modified Willis flotation method to detect parasite eggs using the working procedure with a supersaturated solution based on magnesium sulfate (Cozma and Şuteu, 2004). This method was performed on Days 1, 7, and 14 after treatment administration. The procedure involved mixing approximately 2-3 g of fecal material with 20 ml of supersaturated magnesium sulfate solution in a mortar and pestle until homogenized; filtering the solution through a sieve into a test tube (5-10 ml capacity); resting for 20-30 minutes to allow parasite eggs to float to the surface and placing a coverslip on the surface of the test tube. Faecal smears without staining of both the floated supernatant and the remaining sediment were examined microscopically (10X and 20X) to identify parasite eggs. The adopted coproparasitological testing procedure allowed the selection of relevant preparations for the ovoscopic and larvoscopic evaluation of the evolution of the intensity of parasitism with *Toxocara canis* and *Dypilidium caninum* at 1, 7 and 14 days after treatment.

In quantifying the degree of intensity of parasitism, we resorted to the procedure with conventional symbols used in common clinical assessments (Cozma and Şuteu, 2004), including: - (negative); + (low intensity); ++ (medium intensity); +++ (high intensity); ++++ (very high intensity). The scores given in the initial and final ovoscopic coproparasitological tests were the basis for quantifying the rate of reduction of the helminth egg load of the faeces, more precisely the intensity of parasitism with the two species of gastrointestinal helminths, by adapting the relationship for calculating the oocysts per gram (OPG) reduction, to the specifics of this testing and the scores used, resulting in the following formula:

Reduction in parasitism intensity [%] = initial score-final score/initial score x 100

Hematological Analysis

Blood samples were labeled and examined within 4 hours of collection. A wet mount technique was performed following Smith et al. (2024). This test is intended for the microscopic detection and identification of invasive elements of parasites in various body fluids. Fifthy µL of blood was mixed with 50µL methylene blue solution in an Eppendorf tube. The solution was homogenized, and 4µL was placed in the center of a slide, using an automatic pipette. A coverslip was carefully placed at a 30–45° angle to allow uniform dispersion. The slide was examined under 20x and 40x magnification to detect invasive parasitic forms (mainly larvae in various stages).

Previous Deworming History

Ten dogs were dewormed 3 months prior the study with Drontal tablets (50 mg praziquantel, 144 mg pyrantel embonate and 150 mg febantel) and 6 dogs with fenbendazole (100 mg/kg). Four dogs had no available deworming history. For external parasite control, all 20 dogs had received Fipronil-based treatment one month before the study starting date.

Statistical Analysis

The collected data were subjected to descriptive statis-

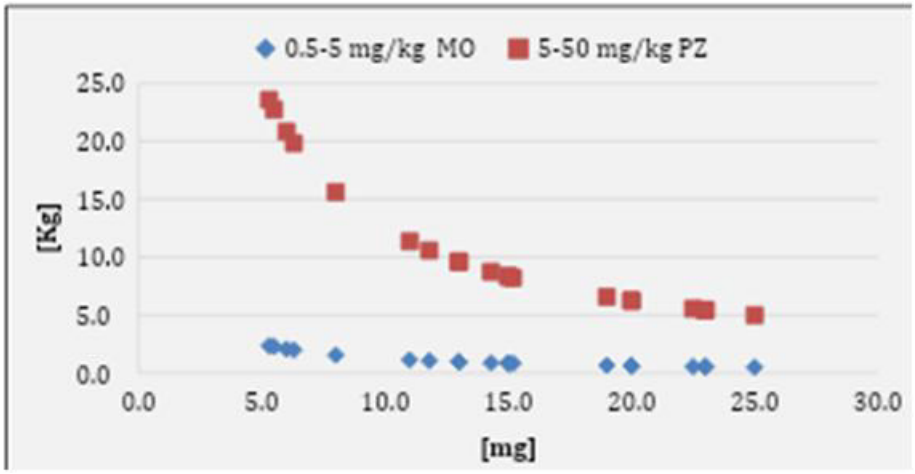


Figure 1. Dose distribution of Milbemycin oxime and Praziquantel in relation to the weight of dogs

tical analysis to determine the following parameters: mean, standard deviation (Std. Dev.), maximum value (Max.), minimum value (Min.) and Interval.

RESULTS AND DISCUSSIONS

Following the evaluation of the dog population the subjects included in this study were selected from an animal shelter (n=20), 20 subjects of common breed (7 males and 13 females). Consequently, the included dogs represent a heterogenous sample from the population with an average age of 3.7 years (1-10 years) and an average bodyweight of 14.5 kg (4-50 kg), were selected. This approach aimed to enhance the generalizability of the findings across different physiological profiles. The results obtained in the field evaluation of the efficacy, safety and palatability of the generic Milbenin formula in the therapy of natural helminthiasis in dogs, confirmed the coproparasitological presence of natural infestations with *T. canis* and *D. caninum* of dogs included in the study, fulfilling the main criterion that was the basis for the selection of subjects. Relevant results were also obtained for the analysis of the effectiveness of the therapeutic procedure adopted, which consisted in the administration of tablets, in accordance with the manufacturer's recommendations (one tablet for a dog with a body weight between 5 and 25 kg). The weights of all patients included in the study fell within this range, each of them being treated with one Milbenin tablet. When analyzing the recommended dosage for the active molecules, it turned out to be 0.5-1 mg/kg Milbemycin oxime and 5-50 mg/kg Praziquantel, falling within the limits of 0.5-2.4 mg/kg for Milbemycin oxime and 5-23.6 mg/kg for Praziquantel. Thus, the average dose was 1.1 mg for Milbemycin oxime and 10.9 mg for Praziquantel with variations related to the weight of the treated dogs, which are relevantly illustrated in the graph in figure 1. Based on the results obtained in the coproparasitological controls performed on day 7 and 14 after the initial treatment, it was decided to repeat the treatment with another tablet of Milbenin only to the 2 patients that were found positive in the coproparasitological control performed 7 days post-therapy.

The detailed analysis of the results is presented in Tables 3 and 4. Out of the 20 dogs infested with *T. canis* and *D. caninum* and enrolled, 18 (90%) were successfully treated (Table 3). The post-therapeutic coproparasitological examinations on days 7 and 14, were parasitologically negative. The exception was the 2 patients (10%) which remained coproparasitologically positive with *D. caninum* at the post-therapeutic examination on day 7, and they were treated with the second therapeutic dose. Finally, at the second coproparasitological control, carried out 14 days after the administration of the first dose, all patients were found negative and were considered clinically cured. The distribution by sex of the 18 dogs successfully treated by administering a single dose included 12/13 females (92.3%), respectively 6/7 males (85.71%), and the application of the normative threshold for characterizing according to age revealed the following distribution: juveniles (0.5-1 years) 87.5%, mature adults (2-6 years) 100%, early seniors (7-9 years) 75%, late seniors (10-18+) 100%. The oral administration procedure of the tablets consisted of direct manual administration, which proved very easy for the entire batch of dogs.

Regarding the results obtained at the initial and final clinical examinations, we note that we did not report any obvious manifestations of the major functions at the screening performed on the day of the group establishment, respectively at the one performed on the 14th day post-therapeutic. Based on the evolution of the values recorded at the monitoring of the basic physio-

Table 3. Summary of the results obtained from parasitological examinations

Pacient (No.)	*Dipylidum caninum*				*Toxocara canis*				Flee	Ticks	Contact*	Deworming**
	Day			Red.	Day			Red.				
	0	7	14	[%]	0	7	14	[%]	++	+++	No	Unknown
1.	+++	++	+	75	+	-	-	100	+	-	Yes	yes
2.	-	-	-	100	+	-	-	100	++	-	yes	yes
3.	-	-	-	100	++	+	-	100	-	-	yes	yes
4.	++	-	-	100	-	-	-	100	++++	●	yes	Yes
5.	-	-	-	100	++	-	-	100	+++	-	yes	Unknown
6.	-	-	-	100	++	+-	-	100	-	-	yes	Unknown
7.	-	-	-	100	++	-	-	100	+++	-	yes	yes
8.	++	++	-	100	++	+	●	- 100	-	-	yes	yes
9.	-	-	-	100	+	-	-	100	++	-	yes	yes
10.	-	-	-	100	++	-	-	100	++	-	yes	yes
11.	-	-	-	100	++	-	-	100	++++	-	yes	yes
12.	+++	++	-	100	-	-	-	100	+++	-	yes	Unknown
13.	++	++	+	75	+	-	-	100	-	-	yes	yes
14.	-	-	-	100	++	-	-	100	++	-	yes	yes
15.	++	-	-	100	-	-	-	100	+++	-	yes	yes
16.	-	-	-	100	+	-	-	100	-	-	yes	yes
17.	-	-	-	100	++	++	●	100	-	+++	yes	yes
18.	-	-	-	100	++	●	-	100	-	-	yes	yes
19.	-	-	-	100	+++	+	-	100	-	-	yes	yes
20.	-	-	-	100	+	-	-	100	++	-	yes	yes
Efficacy rate by gender and age: 90%; females 92,3%, males 85,71%, juveniles 87,5%, adults 100%												

* With other dogs; ** macroscopic changes; ***Previous deworming (3 months prior); - negative; + low intensity; ++medium intensity; +++ high intensity; ++++ very high intensity; Red.- reduction in parasitism intensity [%] [R= initial score-final score/initial score x 100]. **Deworming performed in the last 3 months.

logical parameters, shown in Table 4, we can conclude that the forms of dipylidiasis and toxocariasis detected evolved subclinically, and the anthelmintic therapy with a single dose of Mildemin did not produce any secondary manifestations in any patient.

These findings indicate that the treatment had a substantial effect in reducing infestation rates over time. Quantitative analysis of *Toxocara canis* infestations showed a decline from 71.42% on day 7 to 0% by day 14, while *Dipylidium caninum* infestations decreased from 66.66% to 33.33% over the same period. A considerable number of the dogs were infested with fleas, which are well-established intermediate hosts. This suggests that the parasitic life cycle may not have been fully disrupted, potentially allowing for the continued presence of parasite elements in the feces up to day 14 of the study (Figure 2).Thus, the prevalence of parasitism evolved from 85% on day 1 to 15% on day 7 and 5% on day 14. The sample remaining positive on the last day of the study came from a dog with an unknown deworming history. Subsequently, the analyses of this dog with massive parasitic infestation were completed with the evaluation of the hemato-biochemical profile and the rapid 4dx test (Heartworm, Lyme, Ehrlichia canis, Ehrlichia ewingi, Anaplasma phagocytophilum and Anaplasma platys). A massive eosinphilia was revealed with a normal biochemical profile and a positive test for Anaplasma platys. Figure 2

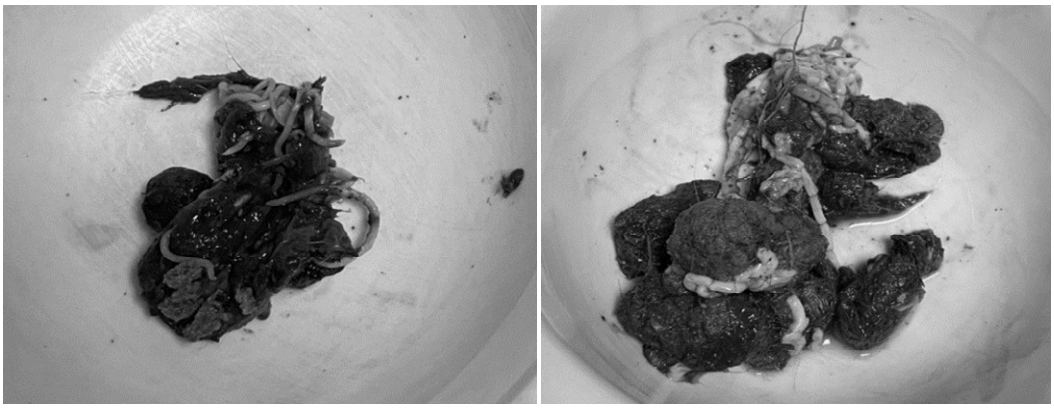
In a similar study, focused on evaluating the efficacy of two anthelmintic products, based on spinosad and milbemycin oxime (Trifexis), and ivermectin and praziquantel (Endovet), only Toxocara-positive dogs were evaluated and efficacy was assessed at 87% and 71%, respectively, after examining samples 14 days after product administration, using testing methods similar to those in our study (Cardenas et al., 2017). Regarding the degree of palatability of the product, we noted the dogs' preference for voluntary consumption of the tablets, easily ingesting the tablets together with a small amount of dry food. The results of the Chi-square test are highlighted in Figure 3 A and B. As shown in the data summarized in Figure 2, canine and feline heartworm disease can be effectively treated with anthelmintics containing the active molecules epsiprantel or praziquantel, often combined with an external antiparasitic agent to expand the spectrum of action and break the parasite's life cycle. Praziquantel is also used to treat dipylidiasis in humans (Chelladurai et al., 2018). Several pharmaceutical products with these active molecules currently have a major impact in anthelmintic therapeutics and prophylaxis in several species of companion and livestock animals (Todoran et al., 2024)

Toxocariasis and dipylidiasis are very common zoonotic parasitic diseases in some areas, including the area taken in this study. The control of these infestations

Table 4. Evolution of basic physiological functions measured at screening and final examination status

Subject code	Screening			Final examination		
	Body temp. (^{0}C)	Pulse (/min)	Resp. (/min)	Body temp. (^{0}C)	Pulse (/min)	Resp. (/min)
1	39	80	11	38.3	90	18
2	39.1	79	20	38.5	100	22
3	39.0	77	17	39	89	31
4	38.9	89	13	39.1	90	16
5	39.2	70	19	38.7	88	13
6	39	90	22	38.5	96	25
7	38.9	88	14	38.4	78	20
8	38.9	85	20	38.9	95	19
9	39	86	14	38.3	87	16
10	39.1	83	15	38.9	85	24
11	39.3	75	35	38.5	93	30
12	39.2	120	33	39.2	100	29
13	38.9	118	34	39.3	120	31
14	39.1	120	21	39	121	33
15	38.8	89	25	38.6	99	26
16	38.9	77	30	38.9	91	17
17	38.9	90	17	39	84	25
18	39.1	91	22	38.3	79	16
19	39.2	118	33	39.1	100	34
20	38.9	87	29	39	110	24
Count	20	20	20	20	20	20
Mean	39	87,5	20,5	38,9	92	24
SD	0,1361	15,64	7,688	0,324	11,70	6,419
Range	38,8-39,3	70-120	11-35	38,3-39,3	78-121	13-34
Minimum	38,8	70	11	38,3	78	13
Maximum	39,3	120	35	39,3	121	34

*Body temperature (average)-102°F (38.9°C); pulse- 70 to 120 beats per minute; Respiratory rate (at rest) 18 to 34 breaths per minute (MSD Veterinary Manual).



A **B**

Figure 2. Macroscopic examination of fecal specimens infested with *T. canis (A) and D. caninum (B)*

has a great impact on the prevention of environmental pollution, in prophylactic-therapeutic deworming protocols and in educational strategies. The zoonotic character is the most worrying fact, which is given by the risk of public contamination and implicitly of affecting human health.

Milbemycin is a less commonly used anthelmintic product in our country, reason for which there are not many publications available on its use in the control of *Toxocara canis* infestations. In this regard, we will briefly recall a study that shows that the treatment with

Table 5. Efficacy of some anthelmintic products in the treatment of infestations with *Toxocara canis* and *Dypilidium caninum*

Antiparasitic products agains *Toxocara canis* in dogs				
Molecule(s)	**Formulation**	**Days**	**Efficacy (%)**	**Reference**
Nitazoxanide	Oral	7	90	Yuan et al., 2024
Selamectin	Topic	14	89.5	McTier et al., 2000
Praziquantel, pyrantel embonate, febantel	Oral	14	98.1	McTier et al., 2000
Milbemycin oxime, Praziquantel	Oral	14	100	Current study
Emodepside, toltrazuril	Oral	7	100	Schimmel et al., 2011
Milbemycin oxime, lotiranel	Oral	14	98.6-96.8	Young et al., 2021
Milbemycin oxie, Spinosad	Oral	14	99.25	Bowman et al., 2014
Ivermectin, praziquantel	Oral	28	88	Cardenas et al., 2017
Fenbendazol, pyrantel embonate, praziquantel	Oral	23	100	Burcáková et al., 2021
Oxantel, pyrantel, praziquantel	Oral	21	98.9	Grandemange et al., 2007
Moxidectin, praziquantel	Oral	10	100	Paliy et al., 2021 a
Milbemycin oxime, praziquantel	Oral	14	100	Current study
Antiparasitic products against *Dypilidium caninum* in dogs				
Afoxolaner, milbemycin oxime	Oral	28	100	Beugnet et al., 2017
Fipronil-amitraz-(S)-methoprene	Topic	60	100	Beugnet et al., 2013
Pyrantel pamoate, praziquantel	Oral	10	100	Paliy et al., 2021 b
Albendazol	Oral	5	100	Paliy et al., 2021 c
Fenbendazol, pyrantel embonate, praziquantel	Oral	7	100	Burcáková et al., 2021
Oxantel, pyrantel, praziquantel	Oral	14	100	Grandemange et al., 2007
Moxidectin, praziquantel	Oral	10	100	Paliy et al., 2021 (3)
Milbemycin oxime, praziquantel	Oral	14	95	Current study

Milbemycin of dogs infested with *Toxocara canis* decreased significantly compared to a control treatment, the efficacy of milbemycin reaching 55.9% in the control of *Toxocara canis* infestation. According to a study on adult sheep, of the Churra breed, 1% injectable and 0.2% oral solutions of moxidectin (with a dose rate of 0.2 mg moxidectin/kg body weight), proved safe and very effective (100%) in the treatment of pulmonary helminthiasis, including that caused by *D. filaria* (Hidalgo et al, 2002). In table 5 it is presents data on the efficacy of various anthelmintic agents in treating canine infestations caused by *Toxocara canis* and *Dipylidium caninum*. Furthermore, Table 6 offers a consolidated overview of studies evaluating the therapeutic effectiveness of milbemycin oxime and praziquantel in dogs.

Praziquantel is a highly effective cestocide drug, and resistance was rarely reported. Between 2016 and 2018, a population of dogs with cestode infestations resistant to Praziquantel and Epsiprantel was identified. These cases did not respond to treatment, even with increased dosages, higher frequency, and prolonged administration. Molecular analysis identified resistant isolates, with genetic mutations in 28S, 12S, and voltage-gated calcium channel genes. The only effective alternative treatments were Nitroscanate or a combination of Pyrantel/Praziquantel/Oxantel. Veterinarians should be aware of this emerging resistance, as treatment options for cestodes are limited in both human and veterinary medicine (Chelladurai, 2018). The findings show the high level of efficacy and safety of the tested anthelmintic formula, as well as the good palatability of the formulated tablets, all of which are real advantages for supporting the use of the generic product Milbenin in the treatment of gastrointestinal helminthic infestations in dogs. In addition to the above, we recall that an innovative or generic medicinal product with good therapeutic efficacy must have a high level of bioavailability and be free of side effects and interactions with other medicines or food (Lainesse et al, 2012).

Milbemycin oxime is a well-tolerated anthelmintic molecule in dogs of all ages. However, very young puppies may be slightly more sensitive to overdoses. Clinical signs usually appear only after a tenfold overdose or higher. Common symptoms observed after extreme overdoses include: reduced activity, unstable gait and lack of coordination, prostration in severe cases. The severity and duration of symptoms were dose-dependent. Tremors, occasionally observed after overdoses, may not always be related to the treatment, as untreated dogs have also exhibited similar reactions. Diarrhea has been observed sporadically but is unlikely to be treatment-related, as its occurrence is inconsistent and not dose-dependent. Affected dogs recovered within a day without additional treatment. Milbemycin oxime is well tolerated by Collies. In Collies, a tenfold overdose of Milbemycin oxime resulted in mild, transient clinical signs such as reduced activity, ataxia, and salivation (Jung et al., 2002).

Table 6. The efficacy studies of the milbemycin oxime (MO) and praziquantel (PZ) in cestodes and nematodes of dog

Active substance	Infestation	Dose/route of administration	Results	Ref.
MO/PZ - Interceptor Plus	Naturally Dipylidium caninum	Orally 0.5 mg/kg MO and 5 mg/kg PZ	Treatment with milbemycin OM/PZ tablets was 100% effective against naturally-acquired adult *D. caninum* tapeworm infections in dogs. The difference in the number of *D. caninum* recovered between the treated and control group was statistically significant.	*Freedom of infor-mation Summary, 2017*
MO/PZ - Milpro®	Orally Ec. multilocularis	2.5 mg MO, 25 mg PZ or 12.5 mg MO, 125 mg PZ	A single oral dose of Milpro® film-coated tablets for dogs and Milpro® film-coated tablets for small dogs and puppies was demonstrated to be a safe and 100 % effective treatment.	*Cvejic et al., 2016*
MO/PZ - Milbemax®	Dipylidium caninum	Orally 0.5 mg/kg M and 5 mg/kg PZ	Milbemax® showed 100% efficacy against all the intestinal helminths (hookworms, Trichuris, Toxocara and Dipylidium) in all the kennels and at each sampling time (Days 7, 14 and 21).	*Rinaldi et al., 2015*
PZ	T. hydatigena T. multiceps Ec. granulosus	5–10 mg/kg PZ	The overall prevalence of taeniid cestodes was reduced from 34.4% at the beginning of the trial to 9.6% and to 4.9% 1- 2 years after starting the trial, respectively. E. granulosus prevalence changed from 13.3% to 3.6% and to 2.4%, T. hydatigena prevalence changed from 20.0% to 2.4% and to 2.4%, and T. multiceps prevalence changed from 1.1% to 3.6% and to 0% 1 - 2 years after starting the trial, respectively.	*Guo, Z., et al., 2013*
PZ	Naturally Dipylidium caninum	Orally, 5 mg/kg bw	Dogs showed marked recovery post treatment. On re-examination after a week, faeces were found negative for any parasitic eggs/gravid egments.	*Saini, V.K., ET AL., 2016*
MO/PZ - Milbemax®	Hookworms Trichuris, Toxocara	Orally 0.5 mg/kg M and 5 mg/kg PZ	Milbemax® showed 100% efficacy against all the intestinal helminths (hookworms, Trichuris, Toxocara and Dipylidium) in all the kennels and at each sampling time (Days 7, 14 and 21).	*Rinaldi et al., 2015*
MO/PZ - Milbemax®	Tr. Vulpis, T. canis, To. leonine A. caninum	Orally 0.5 mg/kg M and 5 mg/kg PZ	The results show high efficacy values of milbemycin oxime plus praziquantel tables against T. vulpis (99.6%), T. canis (99.8%), T. leonina (100%), Ancy. caninum (99.4%) and D. caninum (100%)	*Schimmel et al., 2011*
MO	Trichuris vulpis	Orally,0.5-1.0 mg/kg bw	Milbemycin oxime has shown mean efficacies of 96.0% and 98.6 % at doses of 0.5 mg/kg and 1.0 mg/kg bw, respectively.	*Horii et al., 1998*
MO - Interceptor® Flavor Tabs® Sentinel®	Anchylostoma braziliensis	Orally, 05 mg/kg bw	Statistically, both of the milbemycin oxime treatment groups had significantly ($p < 0.0001$) fewer A. braziliense isolated at necropsy when compared to the placebo control group with reductions ranging from 94.91% (Sentinel) to 98.02% (Interceptor) from a single administration.	*Bienhoff et al., 2013*
MO/PZ - Milbemax®	Thelazia callipaeda	Orally 0.5 mg/kg M and 5 mg/kg PZ	The commercial formulation of MO (MilbemaxW) at the 0.5 mg/kg and 2 mg/kg b.w. for dogs and cats, respectively, also containing PZ (5 mg/kg b.w.), showed a high therapeutic efficacy in curing T. callipaeda in naturally infested. In dogs the efficacy was 72.7% and 90.9% after a single or two treatments, at a weekly interval.	*Motta et al., 2012*
MO - Interceptor®	Naturally aquired Thelazia callipaeda	Orally 0.5 mg/kg MO	A monthly treatment with MO (Interceptor1, Novartis), at the dose recommended for heartworm disease prophylaxis, is also highly effective (96.7%) for the prophylaxis of T. callipaeda.	*Ferroglio et al., 2008*
MO/PZ - Milbemax®	Crenosoma vulpis	MO (0.6 mg/kg per os)	Cough completely disappeared within the next two weeks.	*Caron et al., 2014*

Table 6. Continue

MO	Crenosoma vulpis	Orally, 0.5 mg/kg, single dose	Routine use of monthly parasite control products containing milbemycin oxime or moxidectin are expected to prevent establishment of future infections.	*Bowman., 2014*
MO	Dirofilaria immitis	Orally, 0.05-1.0 mg/kg-5 mg/kg /days 1- 90	No worms were recovered from dogs of all groups in which milbemycin oxime was administered at a dose of 0.25 mg/kg on days 15 to 60 after infection.	*Tagawa et al., 1993*
MO/PZ - Milbemax®	Dirofilaria repens	Orally, 0.5 mg/kg MO + 5 mg/kg PZ	The monthly use of Milbemax® in dogs is effective and safe for the prevention of subcutaneous dirofilariosis in endemic areas.	*Di Cesare et al., 2014*

Praziquantel, when used orally, may cause mild side effects in dogs, but the incidence is less than 5%. Reported side effects include: anorexia, vomiting, lethargy, diarrhea (Badreldin, H., 2006).

CONCLUSIONS

The increase in the prevalence of canine helminthiasis, especially in shelter dogs, in the study area, reveals the need to diversify the prophylactic-therapeutic medication available on the veterinary medical market. In this regard, we believe that our study brings new arguments to support the “in vivo” therapeutic efficacy of Milbemycin oxime and Praziquantel in infestations with *Toxocara canis* and *Dipylidium caninum*. The overall analysis of the results obtained in the clinical evaluation of the new generic product Milbenin, reveals the correlation of very good levels of therapeutic efficacy and tolerance of this anthelmintic. It proves to be a viable alternative to existing canine deworming products and may be safely interchanged with other anthelmintics currently available on the market.

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