

Field study on the efficacy of an oral 2% ivermectin formulation in horses

Eficácia a campo de uma formulação oral de ivermectina a 2% em equinos

André Antonio Cutolo^{1*}; Anderson Tintino dos Santos¹; Silmara Marques Allegretti¹

¹Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP

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Abstract

Twenty horses naturally infected with nematodes were included in a blind, controlled field study on efficacy and safety of an oral 2% ivermectin formulation at a dose of 0.2 mg.kg⁻¹. Horses were divided into treated and non-treated (control) groups with ten animals each based on preliminary counts of eggs per gram of feces (EPG). Stool samples were collected after treatment for identification of nematode species. Clinical evaluations and EPG counts were performed on days 0, +5, +14 and +19. Nineteen nematode species were identified: *Coronocylus ulambajari*, *Craterostomum acuticaudatum*, *Cyathostomum catinatum*, *Cyathostomum pateratum*, *Cylicocyclus brevicapsulatus*, *Cylicocyclus insigne*, *Cylicocyclus leptostomum*, *Cylicocyclus nassatus*, *Cylicocyclus ultrajectinus*, *Cylicocyclus* spp., *Cylicostephanus calicatus*, *Cylicostephanus longibursatus*, *Cylicostephanus poculatus*, *Habronema muscae*, *Habronema* spp., *Parascaris equorum*, *Poteriostomum imparidentatum*, *Oxyuris equi* and *Triodontophorus* spp. The mean EPG counts of treated and non-treated (control) groups on Days -15, 0, +5, +14 and +19 were 1925, 1340, 0, 12.5, 0, 1470, 790, 875, 1605 and 1240 respectively. The efficacy of treatment on Days +5, +14 and +19 was 100, 99.2 and 100% respectively, with a significant difference compared to the control group ($p < 0.01$). The product was considered to be safe with no findings of clinical significant changes during the study.

Keywords: Anthelmintic, EPG, efficacy, dewormer, antiparasitic.

Resumo

Vinte equinos naturalmente infectados com nematódeos foram utilizados em estudo cego, controlado, de eficácia e segurança clínica a campo de uma formulação oral de ivermectina a 2%, na dosagem de 0,2 mg.kg⁻¹. Foram distribuídos em grupos: tratado e sem tratamento, de dez animais cada, baseados na contagem prévia de ovos por grama de fezes (OPG). Amostras de fezes foram colhidas pós-tratamento para identificação da helmintofauna. Avaliações clínicas e OPG foram realizados nos dias 0, +5, +14 e +19. Identificou-se dezenove espécies de nematódeos: *Coronocylus ulambajari*, *Craterostomum acuticaudatum*, *Cyathostomum catinatum*, *Cyathostomum pateratum*, *Cylicocyclus brevicapsulatus*, *Cylicocyclus insigne*, *Cylicocyclus leptostomum*, *Cylicocyclus nassatus*, *Cylicocyclus ultrajectinus*, *Cylicocyclus* spp., *Cylicostephanus calicatus*, *Cylicostephanus longibursatus*, *Cylicostephanus poculatus*, *Habronema muscae*, *Habronema* spp., *Parascaris equorum*, *Poteriostomum imparidentatum*, *Oxyuris equi* e *Triodontophorus* spp.. As contagens médias de OPG dos grupos tratado e controle nos dias -15, 0, +5, +14 e +19 foram respectivamente 1925, 1340, 0, 12,5, 0 e 1470, 790, 875, 1605 e 1240. A eficácia do produto nos dias +5, +14 e +19 foi respectivamente de 100, 99,2 e 100%, com diferença significativa em relação ao grupo controle ($p < 0,01$). O produto mostrou-se seguro, não sendo observadas alterações clínicas dignas de nota durante o experimento.

Palavras-chave: Anti-helmíntico, OPG, eficácia, equino, vermífugo.

*Corresponding author: André Antonio Cutolo
Departamento de Biologia Animal, Instituto de Biologia,
Universidade Estadual de Campinas – UNICAMP,
Cidade Universitária “Zeferino Vaz”, Rua Monteiro Lobato, 255,
CEP 13083-862, Campinas - SP, Brazil;
e-mail: cutoloandre@yahoo.com

Equine endoparasites can cause health damage to the host with clinical signs that include reduced growth rate, abdominal distension, poor body condition, weakness, reduced physical performance and fertility, reduced digestion and nutrient absorption, cramps from intestinal compaction, intestinal rupture and death, among others (BRADY; NICHOLS, 2009).

Anthelmintics are fundamental tools used for endoparasite control and include drugs from the group of benzimidazoles (fenbendazole, oxfendazole, mebendazole and oxibendazole), piperazines, tetrahydropyrimidine (pamoate and pyrantel tartrate) and macrocyclic lactones (ivermectin and moxidectin) (SANGSTER; GILL, 1999; BRADY; NICHOLS., 2009).

Parasitic resistance to anthelmintic drugs is known for different active ingredients against nematode species of several hosts including equines (SANGSTER; GILL, 1999) which also have multiresistant populations (BRADY; NICHOLS, 2009; KAPLAN, 2004).

In horses, Cyathostominae populations resistant to benzimidazoles and pyrantel salts have been reported in different countries (BRADY; NICHOLS, 2009) including Brazil (ALMEIDA et al., 2004). There are also reports of anthelmintic resistance to macrocyclic lactones including ivermectin and moxidectin (BOERSEMA et al., 2002).

The evaluation of reduction in the number of eggs per gram of feces (EPG) is a major test for the clinical diagnosis of resistant helminth populations in equines (KAPLAN, 2002).

Therefore, field efficacy of a new oral 2% ivermectin formulation was assessed in equines naturally infected by nematodes by means of evaluation of EPG reduction, according to the method suggested by Duncan et al. (2003), in addition to the identification of helminth parasite fauna in the study site.

Twenty mixed-breed horses with the highest EPG counts (≥ 200) were selected from a group of 30 animals evaluated on Day -15 out of a total of 65 horses at the study site at baseline. The property is located in the city of Monte Mor (22° 57' 00.63" S and 47° 19' 46.92" W) in the State of São Paulo, Southeastern Brazil, and is dedicated to horse-rearing and trade for over forty years. Two animals from the treated group were sold before the end of the experiment, and only 8 animals remained in this group.

Of the selected animals, 12 were male and eight were female. Two males were stallions and the others were castrated. One female horse was pregnant. Ten animals were foals (1 to 2.5 years old) and the other ten were 3 to 15 years old. Their weight ranged from 290 to 550 kg. None of the selected animals had received any anthelmintic treatment at least 30 days before Day -15.

According to the management protocol adopted by the property, during the study the animals remained in individual stables at night and in collective pasture area with star grass (*Cynodon* spp.) during the day, and received feed produced at the site (oat 20%, milled corn 35%, wheat bran 28%, soy bran 6%, calcium phosphate 10% and oat bran q.s.p.).

On the day before treatment (July 1st, 2009) the animals were individually weighed on a scale (Coimma, manufacture number 13741). On Day 0 (July 2nd, 2009) the animals were individually restrained, their feces and blood collected and then orally treated with the test product (Padock® Gel, Sespo Indústria e Comércio Ltda., Batch 0095P1/09, Manufacture February, 2009), based on 2% ivermectin at a dose of 0.2 mg.kg⁻¹. The animals in the

negative control (non-treated) group were treated with the test product after the end of the experiment on Day +19.

The animals were assessed for clinical and behavioral changes for up to two hours after treatment and afterwards on Days 0, +5, +14 and +19. Samples of approximately 200 g of feces were collected directly from the rectum or from the soil when recently excreted on Days 0, +1, +5, +14 and +19, always in the morning. On Day +1 feces were collected 26 hours after treatment for the assessment of the eliminated nematode specimens. All biological samples collected were stored in an isothermal box, refrigerated and immediately taken to the Universidade Estadual de Campinas (UNICAMP) Laboratory of Helminthology for helminth fauna processing and identification. EPG counts were conducted according to Gordon & Whitlock method (1939) with a minimum detection of 50 EPG of feces. The laboratory analyses were blind, and the samples were coded so as to prevent technical staff from relating the animals to their groups.

The identification of helminths was performed through the evaluation of all specimens collected in a sample of about 20 g of feces of each animal. The helminths were duly separated, washed and fixed in lactophenol for 60 days for clarification of the anatomical structures and then transferred to glycerinated alcohol for later identification with the aid of an optic microscope, according to Vicente et al. (1997) and Lichtenfels et al. (2008).

The efficacy of the formula was assessed using the percentage of EPG reduction according to the Equation 1:

$$\text{Treatment efficacy} = \left(\frac{\text{Mean number of EPG in the control group}}{\text{Mean number of EPG in the control group} - \text{Mean number of EPG in the treated group}} \right) \times 100 \quad (1)$$

The treatment efficacy was assessed at every trial.

The variables were statistically analyzed using GraphPad InStat v. 3.05, considering a level of significance of 5% ($\alpha = 0.05$). The inter-group efficacy was assessed using the Mann-Whitney's non-parametric test. The intra-group analyses were conducted using the Friedman's non-parametric test and Dunn's multiple comparisons test when a significant difference was detected ($p < 0.05$). For EPG analyses where a standard deviation of zero was obtained, a parametric t-test was used.

The homogeneity of the groups regarding weight was confirmed through the Wilcoxon non-parametric test for unpaired samples, and no significant difference was found between both groups studied ($p = 0.4272$), with mean weight of 372.1 and 376.8 kg in the treated and non-treated groups.

As for EPG assessments, the results obtained on Day -15 allowed the selection of 20 animals with the highest nematode infection levels (mean EPG counts of 1925 for the treated group and 1470 for the control group). There was no significant statistical difference between EPG counts on Day -15 between both groups studied ($p = 0.5288$).

The mean EPG counts are shown in Table 1. Considering that an EPG count of 200 is already considered indicative of infection requiring anthelmintic treatment (PÉREZ et al., 2003),

Table 1. EPG count results for non-treated and treated horses with an oral 2% ivermectin formulation at a dose of 0.2 mg.kg⁻¹.

	Non-treated					Treated				
	Day -15	Day 0	Day +5	Day +14	Day +19	Day -15	Day 0	Day +5	Day +14	Day +19
Total EPG counts	14700	7900	8750	16050	12400	19250	13400	0	100	0
Mean EPG counts	1470 ^A	790 ^A	875 ^A	1605 ^A	1240 ^A	1925 ^A	1340 ^A	0 ^B	12.50 ^B	0 ^B
Std Deviation (SD)	1516.98	1151.76	575.06	1022.65	940.09	1989.31	1312.29	0	23.15	0
Efficacy (%)	-	-	-	-	-	-	-	100.00	99.22	100.00

EPG - eggs per gram of feces. Means followed by the same letter do not differ among them in the inter-group comparison ($p \geq 0.05$).

the level of parasites of the animals was considered adequate for conducting the study.

In the analyses conducted on Day -15, the presence of *Parascaris equorum* eggs were observed in one animal in the treated group. After treatment with 2% ivermectin, eggs from this species were no longer found in the animals, indicating efficacy of the treatment for egg elimination.

The count results obtained on Day 0 were also similar in both groups with no statistical differences ($p = 0.1506$), reflecting homogeneous infection before randomization.

On Days +5, +14 and +19 there was seen a dramatic reduction in EPG counts in the treated group with results equal to zero in all the animals on Days +5 and +19, and EPG count was 50 in only two positive animals on Day +14. There was a significant difference between the control and treated groups, p -values of 0.0017, <0.0001 and 0.0043 respectively.

The efficacy rates on Days +5, +14 and +19 were 100, 99.22 and 100% respectively.

As for the control group, there was no significant intra-group difference among the different days of assessment (except in the comparison between Day 0 and Day +14), which reflects homogeneous infection in the animals, and it remains unchanged throughout the entire study duration.

As shown in Table 2, the following nematode species were identified in the feces of the animals studied within 26 hours post-treatment: *Coronocylus ulambajari*, *Craterostomum acuticaudatum*, *Cyathostomum catinatum*, *Cyathostomum pateratum*, *Cylicocyclus brevicapsulatus*, *Cylicocyclus insigne*, *Cylicocyclus leptostomum*, *Cylicocyclus nassatus*, *Cylicocyclus ultrajectinus*, *Cylicocyclus* spp., *Cylicostephanus calicatus*, *Cylicostephanus longibursatus*, *Cylicostephanus poculatus*, *Habronema muscae*, *Habronema* spp., *Parascaris equorum*, *Poteriostomum imparidentatum*, *Oxyuris equi* and *Triodontophorus* spp.

A total of 881 helminth specimens belonging to 19 different species were recovered through the examination of the samples of feces. Of these, 526 (59.7%) were pre-selected for identification due to good preservation, being 221 (42%) male and 305 (57.9%) female. Of the 526 identified specimens, 501 (95.2%) belonged to the subfamily Cyathostominae, which was the group with the highest prevalence in the local helminth fauna.

The most abundant species were *Cylicostephanus longibursatus*, *Cyatostomum catinatum*, *Cylicocyclus leptostomum* and *Cylicostephanus calicatus*, found in 100, 90, 90 and 70%, respectively, of the animals examined. These four species together accounted for 85.93% of the 526 specimens collected and identified, and *C. catinatum* and *C. leptostomum* were the most abundant with over 50% of the total identified, 33.65 and 28.13% respectively.

Table 2. Identified species, and number and abundance of nematode specimens in horses treated with an oral 2% ivermectin formulation at a dose of 0.2 mg.kg⁻¹.

Nematode Species	n	Abundance (%)
<i>Cyatostomum catinatum</i> (Looss, 1900)	177	33.65
<i>Cylicocyclus leptostomum</i> (Kotlan, 1920)	148	28.13
<i>Cylicostephanus longibursatus</i> (York & Macfie, 1918)	89	16.92
<i>Cylicostephanus calicatus</i> (Looss, 1900)	38	7.22
<i>Poteriostomum imparidentatum</i> (Quiel, 1919)	23	4.37
<i>Cyathostomum pateratum</i> (York & Macfie, 1919)	14	2.66
<i>Parascaris equorum</i> * (Goeze, 1782)	4	-
<i>Cylicocyclus nassatus</i> (Looss, 1900)	3	0.57
<i>Triodontophorus</i> spp. (Looss, 1900)	3	0.57
<i>Cylicocyclus ultrajectinus</i> (Ihle, 1920)	2	0.38
<i>Cylicostephanus poculatus</i> (Looss, 1900)	2	0.38
<i>Cylicocyclus</i> spp. (Ihle, 1922)	2	0.38
<i>Cylicocyclus insigne</i> (Boulenger, 1917)	1	0.19
<i>Cylicocyclus brevicapsulatus</i> (Ihle, 1920)	1	0.19
<i>Craterostomum acuticaudatum</i> (Kotlán, 1919)	1	0.19
<i>Coronocylus ulambajari</i> (Dvojnjos et al., 1994)	1	0.19
<i>Oxyuris equi</i> (Schränk, 1788)	1	0.19
<i>Habronema muscae</i> (Carter, 1861)	1	0.19
<i>Habronema</i> spp.	1	0.19

*Eggs identified during EPG assessments on Day -15 with no recovery of adult specimens in feces.

Critical controlled studies conducted during the development of the ivermectin molecule showed its effectiveness against several equine nematode species, including migratory stages in tissues of *Parascaris equorum*, *Strongylus* spp., stomach spiruridae and microfilariae of *Onchocerca cervicalis* (EGERTON et al., 1981).

When compared to other active ingredients available in the market, ivermectin is considered a complete drug for the control of different nematode species and *Gasterophilus* species at the dose of 0.2 mg.kg⁻¹. It is also effective against *Parascaris equorum*, *Strongylus* spp. (adults and immature stages), cyathostominae adults and encysted larvae, *Oxyuris equi*, *Triodontophorus* spp., *Trichostrongylus* spp., *Strongyloides* spp., *Onchocerca* spp., *Habronema* spp., *Draschia* spp., *Dictyocaulus* spp. (BOWMAN et al., 2006).

Although there have been reports of anthelmintic resistance against this active ingredient (BOERSEMA et al., 2002), the results of the present study in naturally infected equines showed the efficacy of ivermectin for helminth infection reduction in horses using EPG count, as reported by other authors (NOGUEIRA et al., 2002; ARAÚJO et al., 2008; SARTORI FILHO et al., 1993; PICHÉ et al., 1991; TAYLOR; KENNY, 1995; CAMPOS PEREIRA et al., 1991). It can be thus confirmed that ivermectin is still a good alternative in equine health programs once the susceptible populations are identified in a horse herd.

The technique used in this study, i.e., determination of equine helminth fauna through the recovery of specimens in feces after anthelmintic treatment, has been previously reported by other authors (OSTERMAN LIND et al., 2003; KUZMINA et al., 2005) and proved feasible.

There is a correspondence between the identification of helminth species eliminated in feces and of those from specimens obtained through necropsy (KUZMINA et al., 2005) with the advantage of lower cost due to non-requirement of horse euthanasia, the possibility of assessing young animals and/or breeders that are not normally sent to the slaughter house and investigating larger groups of animals with known life histories (OSTERMAN LIND et al., 2003).

Seventeen different nematode species in the horse herd examined were identified at the species level, in addition to two others at generic level. The diversity of species was expected to be greater than that found in this study, but some specimens were only identified at generic level and fecal samples collected were small. This would not have occurred if all the specimens of helminths eliminated were collected and identified, as pointed out by other authors (CHAPMAN et al., 2003). The highest abundance of Cyathostominae in the helminth fauna studied is a common finding as this group of helminths can reach a high number per host (SARTORI FILHO et al., 1993; CHAPMAN et al., 2003; KUZMINA et al., 2005; PEREIRA; VIANNA, 2006) with over 50 species belonging to 13 different genera already described (OSTERMAN LIND et al., 2003).

The absence of nematode eggs after the anthelmintic treatment indicates vermifugal action in intraluminal stages of the strongylidae present in the hosts. Despite reports of resistance against ivermectin in equines (SANGSTER; GILL, 1999; BRADY; NICHOLS, 2009), the helminth population examined in this study was susceptible to treatment with an orally administered product at the dose of 0.2 mg.kg⁻¹, as indicated by the manufacturer.

As reported by other authors (SARTORI FILHO et al., 1993), oral ivermectin proved to be safe in the treated animals with no significant clinical changes seen during the experiment.

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