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Short communication

Time required by different anthelmintics to reach expected efficacy levels in horses infected by strongyles

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ABSTRACT

The aim of this study was to determine the time required by different anthelmintic agents to reduce strongyle egg shedding in horses. Fifty horses were divided into five homogenous groups based on faecal egg counts (FECs). Treatment groups received either ivermectin; moxidectin; fenbendazole; piperazine; or no treatment (control group). Faecal examinations were performed 4, 8, 12, 18, 24, 36 and 48 h after the anthelmintic treatment. After this period, faecal samples were taken every 24 h over the next 12 days and finally on alternate days (48-h intervals) for another 14 days until the end of the experiment (28 days post-treatment). The faecal egg count reduction (FECR) was calculated based on the post-treatment mean FECs in the controls and treated animals. Eggs were absent from the faecal examinations beginning at 72 h and 4 days, respectively, following treatment with moxidectin or ivermectin. Piperazine showed an FECR greater than 95% from 48 h up to 9 days post-treatment, with the highest FECR value recorded at 7 days post-treatment (98.1%). However, the FECR was lower than 90% in the last two samplings (26 and 28 days post-treatment). The febendazole group presented the lowest efficacy with FECR below 90% in all samplings. The faecal cultures showed that at the beginning of the trial, all of the groups presented with mixed infections and were predominantly composed of cyathostomins (92.8%), followed by *Strongylus vulgaris* (5.6%) and *Triodontophorus serratus* (1.6%). Only cyathostomin larvae were identified following treatment with fenbendazole or piperazine. In conclusion, horses in the present study had a segment of the cyathostomin population with resistance to fenbendazole and piperazine. The strongyle population was susceptible to macrocyclic lactones, with cessation in egg shedding three and four days after treatment with moxidectin and ivermectin, respectively.

1. Introduction

The Brazilian horse population has been stable over the last decade with approximately 5,514,250 animals (IBGE, 2010). Among all of the factors that should be taken into account in relation to equine health, parasitism has a prominent position. Depending on the worm burden, helminths can have effects ranging from slight abdominal discomfort to poor growth, weight-loss and clinical disease. Gastrointestinal helminth parasites are ubiquitous in grazing horses, with the most important being cyathostomins (small strongyles), *Parascaris equorum*, *Anoplocephala perfoliata* and *Strongylus vulgaris* (Andersen et al., 2013). Anthelmintics are generally considered practical, efficient and safe for the prophylaxis of helminth infections. Among them, benzimidazoles and macrocyclic lactones are the most frequently used in horses raised in Brazil (Spinosa et al., 2002; Molento, 2005).

Egg counts in faecal samples reflect the presence of adult worms in the intestinal lumen. However, the major pathology associated with strongyle infection is typically caused by migrating or encysted larvae (Andersen et al., 2013). Yet faecal examination remains the most useful tool to determine the degree of infection and anthelmintic efficacy, thereby giving support to potential alterations in the prophylactic timetable (Kaplan and Nielsen, 2010).

Other important information is the time required for the elimination of the nematode infection after the anthelmintic treatment. Because such information is very limited in horses, the objec-

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tive of the study was to determine the time required by different anthelmintic agents to reduce strongyle egg shedding in horses based on nematode faecal egg counts (FECs).

2. Materials and methods

This study was performed on the experimental farm of the São Paulo Agribusiness Technology Agency (Agência Paulista de Tecnologia dos Agronegócios, APTA), which is located in the municipality of Andradina, in western São Paulo state, Brazil. We used fifty crossbred (Mangalarga x Quarter Horse) three- to eight-year-old adult horses with an average weight of 350 kg. The horses were kept for 12 months without any anthelmintic treatment before the present study. They were naturally infected by gastrointestinal nematodes and had FECs higher than 150 eggs per gram (EPG). Throughout the experiment, the animals were kept in paddocks with *Panicum maximum* grass and received a mineral mixture and water ad libitum.

On the first day of the experiment, faecal samples were collected directly from the rectum of each horse to determine the EPG using a modified McMaster technique with a detection threshold of 25 EPG (Canever et al., 2013). According to the EPG counts and animal category (sex and age), the horses were distributed into five homogenous groups of 10 animals each with four females and six males in each group. Treatment groups received either fenbendazole (Panacur®, Intervet); ivermectin (Eqvalan®, Merial); moxidectin (Equest®, Fort Dodge); piperazine (Proverme®, Tortuga); or no treatment (control group). The drugs were administered orally at the doses recommended by the manufacturer of each product according to the weight of each animal.

FECs were performed 4, 8, 12, 18, 24, 36 and 48 h post-treatment. After this period, further faecal samples were taken every 24 h for 12 days and then on alternate days (every 48 h) for an additional 14 days until the end of the experiment (28 days after the treatment).

Composite faecal cultures for production of third stage larvae were also performed for each group at each sampling time. Larvae identification was based on the descriptions by Bevilaqua et al. (1993).

The faecal egg count reduction (FECR) was calculated based on post-treatment mean FECs in controls (C) and treated (T) animals according to the following formula: $FECR = 100 \times (1 - (T/C))$. The upper and lower confidence interval limits (95%) were calculated according to methods described by Coles et al. (1992). FEC data were analysed using one-way ANOVA. Data were log-transformed ($\log_{10}(x+1)$) prior to analysis and significant differences between the group means were determined by Tukey's test at a significance level of 5%.

3. Results

All groups presented a similar mean EPG at the time of the treatment: 998 EPG in the Control group, 988 EPG in the Fenbendazole group, 953 EPG in the Ivermectin group, 953 EPG in the Moxidectin group and 935 EPG in the Piperazine group. In comparison with the Control group, a significant reduction in mean FEC was recorded 24 h after treatment with piperazine and 48 h after treatment with fenbendazole, ivermectin or moxidectin ($P < 0.05$). Such significant differences persisted until the end of the trial.

There was a progressive decline in FEC that resulted in the absence of eggs in the faecal examinations at 4 days and 72 h after treatment with ivermectin and moxidectin, respectively. After this time, no more eggs were detected in those groups until the end of the trial. Therefore, these macrocyclic lactones demonstrated 100% efficacy (Table 1).

Table 1
Mean number of eggs per gram of faeces (EPG) of the control group and faecal egg count reduction (FECR) after treatment of horses with fenbendazole, ivermectin, moxidectin or piperazine.

Time[a]	EPG – Control	% FECR			
		Fenbendazole	Ivermectin	Moxidectin	Piperazine
4 h	1844	19.2 (−42; 54)	34.6 (−24; 65)	22.6 (−62; 63)	65.3 (40; 80)
8 h	1845	−3.1 (−104; 48)	28.3 (−45; 64)	22.0 (−50; 59)	64.9 (29; 83)
12 h	1898	10.5 (−73; 54)	9.5 (−53; 46)	25.4 (−48; 62)	63.0 (27; 81)
18 h	1493	1.3 (−89; 48)	22.1 (−38; 56)	18.3 (−57; 57)	59.3 (19; 79)
24 h	940	24.7 (−75; 68)	39.9 (−11; 67)	31.9 (−23; 62)	70.2 (44; 84)
36 h	1015	75.6 (55; 87)	80.3 (59; 91)	68.7 (42; 83)	91.4 (76; 97)
48 h	478	83.7 (67; 92)	96.9 (89; 99)	91.6 (80; 96)	96.3 (92; 98)
60 h	580	83.6 (62; 93)	99.1 (96; 100)	97.8 (91; 99)	96.6 (90; 99)
72 h	694	86.7 (72; 97)	99.3 (97; 100)	100.0	96.8 (79; 100)
84 h	944	85.3 (68; 93)	99.5 (98; 100)	100.0	97.6 (91; 99)
4 d	758	83.5 (66; 92)	100.0	100.0	96.0 (83; 99)
5 d	875	81.1 (62; 91)	100.0	100.0	97.7 (81; 100)
6 d	893	78.7 (66; 87)	100.0	100.0	97.5 (84; 100)
7 d	1033	80.9 (71; 88)	100.0	100.0	98.1 (91; 100)
8 d	958	83.3 (70; 91)	100.0	100.0	96.1 (83; 99)
9 d	1570	84.2 (66; 93)	100.0	100.0	95.2 (85; 98)
10 d	1998	84.1 (68; 92)	100.0	100.0	93.5 (79; 98)
11 d	1415	79.2 (55; 90)	100.0	100.0	93.3 (81; 98)
12 d	1365	72.2 (44; 86)	100.0	100.0	91.6 (72; 98)
13 d	1540	77.9 (56; 89)	100.0	100.0	92.5 (77; 98)
14 d	1620	82.7 (61; 92)	100.0	100.0	94.8 (80; 99)
16 d	1375	83.3 (60; 93)	100.0	100.0	93.8 (82; 98)
18 d	1500	80.9 (57; 91)	100.0	100.0	93.7 (77; 98)
20 d	2150	78.7 (52; 91)	100.0	100.0	92.8 (78; 98)
22 d	2189	70.1 (51; 90)	100.0	100.0	94.0 (82; 98)
24 d	1422	80.3 (56; 91)	100.0	100.0	92.1 (79; 97)
26 d	1853	73.0 (42; 87)	100.0	100.0	89.5 (68; 97)
28 d	1450	73.8 (41; 88)	100.0	100.0	85.9 (64; 95)

FECR (%) = 100 × (1 − treated group mean EPG/control group mean EPG).
Lower and upper confidence interval limits (95%) are in parentheses.
[a] Time after treatment: h (hours) and d (days).

Piperazine showed FECR higher than 95% from 48 h up to 9 days post-treatment (Table 1), with the highest FECR value (98.1%) at 7 days post-treatment. Then, the FECR remained above 90%, excepting the last two samplings, when the values were 89.5% and 85.9%, respectively, at 26 and 28 days post-treatment. Febendazole had the lowest efficacy with FEC means higher than 240 EPG from 9 up to 28 days post-treatment. This group presented the highest value of FECR (86.7%) at 72 h post-treatment (Table 1).

The faecal cultures showed that at the beginning of the trial, the animals in all of the groups presented mixed infections predominantly composed of cyathostomins (92.8%) followed by *Strongylus vulgaris* (5.6%) and *Triodontophorus serratus* (1.6%). These parasites continued to be identified in the control group until the end of the trial, whereas only cyathostomin larvae were identified following treatment with fenbendazole or piperazine.

4. Discussion

Our results corroborate the recommendation by Kaplan and Nielsen (2010) that FECR test (FECRT) should be conducted with faecal samples taken 14 days after treatment. This period is important because benzimidazoles and milbemycins might cause temporary suppression of egg-laying, thus leading to overestimation of the anthelmintic efficacy if the evaluation is carried out during early post-treatment days (Coles et al., 2006; Lyons et al., 2008). This appeared to be also the case of the group treated with piperazine that presented a FECR higher than 95% from 48 h and up to nine days after treatment. Following this period, there was an increase in FEC associated with a reduction in the FECR values (<95%), thus indicating that juvenile cyathostomins survived treatment and reached patency at the end of the trial. A higher efficacy for piperazine has been reported in horses raised in Brazil with FECR of 96% at 14 days- and 91% at 30 days post-treatment (Pereira et al., 1991). Therefore, our results indicate emergence of resistant cyathostomins.

We also observed resistance to fenbendazole in a segment of the cyathostome population. Resistance to benzimidazoles is widespread in horses around the world (reviewed by Peregrine et al., 2014) and also in Brazil (Pereira et al., 1991; Molento et al., 2008; Canever et al., 2013). In contrast, macrocyclic lactones presented 100% efficacy in the tested population of strongyles based on the FECRT carried out up to 28 days post-treatment. Similar efficacies for ivermectin and moxidectin have been reported in other studies with horses (Pereira et al., 1991; Lester et al., 2013). There is evidence that a reduction in the egg reappearance period (ERP) can be an early indicator of resistance to macrocyclic lactones in horses (von Samson-Himmelstjerna et al., 2007; Lyons et al., 2008; Rossano et al., 2010). Therefore, besides the FECRT, it will also be interesting to conduct further studies to determine the ERP following the treatment of the horses with ivermectin and moxidectin.

The reduction in egg shedding was relatively rapid after administration of macrocyclic lactones. Three days after the treatment with moxidectin and four days after the treatment with ivermectin, the FECR was 100% for both treatments, and this level was maintained until the end of the experiment. Due to the fast efficacy of ivermectin and moxidectin in horses, these anthelmintics could be recommended for animals in quarantine before their introduction into the farm. Our results indicate that four days after the anthelmintic treatment might be sufficient to prevent the introduction of new strains of strongyles. Lack of quarantine among recently introduced animals is a management failure that may lead to the introduction of new parasitic strains onto the farm (Torres-Acosta and Hoste, 2008).

In conclusion, horses infected with a susceptible population of strongyles stop shedding eggs in their faeces three and four days after treatment with moxidectin and ivermectin, respectively. The presence of cyathostomins with resistance to fenbendazole and piperazine was recorded.

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