

STUDY ON ANTHELMINTIC EFFICACY OF PRODUCTS BASED ON IVERMECTIN, FENBENDAZOLE AND PYRANTEL IN CONTROLLING DIGESTIVE PARASITOSIS IN EQUINES FROM N-E AREA OF ROMÂNIA, USING FECRT TEST

Costică Toader COVAȘĂ, Liviu Dan MIRON
U.Ș.A.M.V., Facultatea de Medicină Veterinară – Iași
costica_covasa@yahoo.com; livmiron@yahoo.com;

Abstract

Digestive helminths represent an important cause of the equine pathology, among these ones the most representative are the nematodes, from the Strongylidae family, Parascaris equorum and the cestodes such as Anoplocephala perfoliata. The measures to control these parasitosis are mainly based on regular deworming programs using different antiparasitic compounds. There are numerous studies, both nationally and internationally certifying a wider or narrower spread of resistance to some of the substances used for this purpose, especially in some species strongyls and other digestive helminths. The efficacy of treatment with products used in this study was tested on a herd of 56 horses copies of the territory Iași, Suceava, Bacău and Neamț, and the test used was the FECRT. The results showed a high efficiency of all three antiparasitic substances. In the case of ivermectin, FECRT average values were between 98.33% and 100%, for fenbendazole average was 97.11% and 98.21% for pyrantel. Thus, it found a high degree of safety regarding installation chemoresistant, at least for a certain period.

Key words: horses, digestive parasitosis, treatment efficacy, chemoresistance

Introduction

Horses can be infested with over 150 species of intern parasites (Güiris *et al.*, 2010). Intestinal helminths represent an important cause of the equine pathology, among these ones the most representative are the nematodes, from the *Strongylidae* family, and the cestodes such as *Anoplocephala perfoliata* (Osterman Lind *et al.*, 2007; Bonneau *et al.*, 2009). The digestive pests can alter behavior, fertility, physical condition, foals' growth, immunity deficiency to other pathogen organisms or performance aptitudes (Cernea, 2008).

The large strongyles and the cyathostomins are among the world's most significant parasites that infect horses (Riccardo *et al.*, 2010). Among these, recently, the greatest attention has been given to cyathostomins due to the fact that they are considered the main intestinal parasites of horses, with a colossal spread (Morariu *et al.*, 2007; McWilliam *et al.*, 2010). However, strongyls carried out on hosts a variety of pathogenic actions, especially given to large number of parasite species and to many stages of development taking place in the same host in different body tissues and organs. Sometimes there is a high percentage of morbidity and even mortality, especially among youth treated, thus causing multiple damages of medical, economic and animal welfare concerns (Covașă, 2011).

Internationally, the study of equine strongylidosis is a topical issue, particularly regarding the implementation of effective measures to control these morbid entities. Antihelminthic treatments are the basis for these purpose, so that it requires ongoing assesment of results obtained following administration of antihelminthic substances (Covașă *et al.*, 2012).

There are numerous studies, both domestically and internationally, proving a wider or narrower spread of resistance by the parasite involved. Therefore, in this study we aimed to evaluate the effectiveness of commonly used anthelmintic substances in combating digestive parasitosis of horses from N-E region of România, namely ivermectin, fenbendazole and pyrantel, to detect the phenomenon of chemoresistence. For this, we resorted to using qualitative and quantitative coproparasitological techniques, respectively FECRT test (*Faecal egg count reduction test*).

Material and methods

Observations on the effectiveness of treatment with antiparasitic substances were conducted on a herd of 56 horses copies of the territory Iași, Suceava, Bacău and Neamț. Three groups are represented by the following horse farms: livestock farms Rediu, Iași - 6 animals, Rădăuți Stud - 30 animals and Sports Club Blăgești, Bacău - 7 animals. Animals in the last batch, the number 13, came from extensive system of Iași county, the villages Popești and Sinești.

Treatment effectiveness was verified in a comparative way for the following antiparasitic substances: ivermectin, fenbendazole and pyrantel (as pamoate) in combating *Strongylidae* infestations. For this purpose, the test used was FECRT (*Faecal egg count reduction test*) after treatment.

Efficacy of ivermectin was checked in horses from two lots: Stud farm livestock Rădăuți and Rediu. In the first group, antiparasitic product was used as an oral paste, *Noromectin* 1.87 % at a dose of 200 mcg/kg. Rădăuți stud horses were dewormed with *Ecvirom-I* product in the form of oral suspension containing ivermectin 0.2 g/100 ml (1 g/50 ml syringe), and praziquantel 2.5 g/100 ml (1.25 g/50 ml syringe), the ivermectin dose administrated being the same, respectively 0.2 mg/kg.

The fenbendazole effectiveness has been verified in horses from the extensive system in Iași county, following administration of the product *Panacur* oral paste (18.75 %) at a dose of 7.5 mg/kg.

In the lot consists from horses of Sports Club Blăgești, Jud. Bacău, it was tested the effectiveness of pyrantel pamoate, using the product *Pyratel EQ* oral paste containing 132 mg/mL of pyrantel pamoate, the dose administrated being 6.6 mg/kg.

The treatments were performed in the morning and from each animal were harvested samples (approximately 150 g each) from the feces freshly removed the day before administration of the substance antiparasitic (day 0), and in the near future, namely 24 hours, then 3, 5, 7 and 14 days.

To identify the *Strongylidae* parasitic elements, we used the Willis method, as a ovoscopic method, and Baermann, as a larvoscopic method. As a quantitative method, MacMaster method was used to determine the parasitic load for each exemplary, pre- and post-treatment, respectively O.P.G. (eggs/gram of feces).

Subsequently, based on O.P.G. values, it was done the *faecal egg count reduction test*-FECRT. Anthelmintic efficacy of the product was calculated using the formula:

$$E \% = (\text{OPG before treatment (day 0)} - \text{OPG on day 14}) / \text{OPG on day 0} \times 100$$

Results and discussions

Based on coproparasitological analysis, we identified *Strongylidae* parasitic elements, namely eggs in various evolution stages and different sizes and L3 infestant larvae.

In horses from the groups treated with ivermectin, the mean values of the parasitic load degree of pre-treatment day (day 0) were between 50 and 800 O.P.G. Approximately 24 hours after antiparasitic drug administration were obtained higher values, between 150 and 1200, thereafter decreasing gradually, all the values are negative on day 7.

Following administration of ivermectin is observed intensification of eggs removal immediately after treatment, as an effect of antiparasitic substance, followed by progressive decrease in the number of eggs in feces. Negative values of O.P.G. occurred since the 5th day post-treatment, on day 14 the eggs recurrence was observed in the faeces.

The FECRT results after treatment with ivermectin are shown in Table 1 and Table 2.

Table 1.

FECRT values obtained after treatment with ivermectin in horses from Rădăuți Stud					
Crt. no.	Mean OPG day 0	Mean OPG day 7	FECRT %	Mean OPG day 14	FECRT %
1.	150	0	100	0	100
2.	200	0	100	0	100
3.	200	0	100	0	100
4.	350	0	100	0	100
5.	250	0	100	0	100
6.	300	0	100	50	83.33
7.	200	0	100	0	100
8.	200	0	100	0	100
9.	50	0	100	0	100
10.	650	0	100	0	100
11.	150	0	100	0	100
12.	200	0	100	0	100
13.	200	0	100	0	100
14.	350	0	100	50	83.33
15.	250	0	100	0	100
16.	100	0	100	0	100
17.	550	0	100	0	100
18.	300	0	100	0	100
19.	150	0	100	0	100
20.	200	0	100	0	100
21.	300	0	100	0	100
22.	400	0	100	50	83.33
23.	450	0	100	0	100
24.	350	0	100	0	100
25.	100	0	100	0	100
26.	150	0	100	0	100
27.	200	0	100	0	100
28.	150	0	100	0	100
29.	200	0	100	0	100
30.	250	0	100	0	100
Average	251.66	0	100	5	98.33

The obtained results demonstrate a good activity of ivermectin in the treatment of strongylidosis, in the first group FECRT average values showing a 100% efficiency (Covașă *et al.*, 2012), and in the second, a 98.33% efficiency.

Recorded data show a low probability of resistance installation, FECRT values being much higher than limit of 85% which could lead to suspicion emerging phenomenon of resistance.

Table 2.

FECRT values obtained after treatment with ivermectin in horses Livestock farms Rediu

Crt. no.	Mean OPG day 0	Mean OPG day 7	FECRT %	Mean OPG Ziua 14	FECRT %
1.	800	0	100	0	100
2.	500	0	100	0	100
3.	450	0	100	0	100
4.	350	0	100	0	100
5.	350	0	100	0	100
6.	100	0	100	0	100
Average	425	0	100	0	100

The FECRT results for fenbendazole treatment are shown in table 3.

Table 3.

FECRT values obtained after treatment with fenbendazole

Crt. no.	Mean OPG day 0	Mean OPG day 7	FECRT %	Mean OPG Day 14	FECRT %
1.	350	0	100	0	100
2.	400	0	100	50	87.5
3.	700	0	100	0	100
4.	400	0	100	0	100
5.	450	0	100	0	100
6.	550	0	100	0	100
7.	500	0	100	0	100
8.	650	0	100	50	87.5
9.	400	0	100	0	100
10.	750	0	100	50	87.5
11.	550	0	100	0	100
12.	400	0	100	0	100
13.	400	0	100	0	100
Average	500	0	100	11.53	97.11

It is noted the same trend of O.P.G., negative values recorded from the 5th day after the treatment, on day 7 all the samples being negative. The obtained result of FECRT, 97.11 %, show also for fenbendazole a good efficiency without manifestations of the phenomenon of resistance.

Table 4 shows the dynamics of *Strongylidae* egg coproeliminations in horses treated with pyrantel and FECRT values obtained from them.

Also in the case of pyrantel, the results obtained from treated horses show good efficacy, the mean value of FECRT touching 98.21 % of percentage.

It thus appears that the three antiparasitic substances used to control the horses strongylidosis of these lots shows a high degree of safety regarding resistance installation, their rational use in the future ensuring the opportunity of effective control of these parasitosis.

Table 4.

FECRT values obtained after treatment with pyrantel					
Crt. no.	Mean OPG day 0	Mean OPG day 7	FECRT %	Mean OPG day 14	FECRT %
1.	350	0	100	0	100
2.	400	0	100	0	100
3.	700	0	100	0	100
4.	400	0	100	0	100
5.	450	0	100	0	100
6.	550	0	100	50	87.5
7.	500	0	100	0	100
Average	185.71	0	100	7.14	98.21

Worldwide, anthelmintic therapy, especially the strongylidosis, faced with the increasing emergence of chemoresistant populations to probenzimidazole and benzimidazole derivatives, tetrahydropyrimidines even to macrocyclic lactones (Moore, 2000). Resistance to benzimidazole has been reported in North America, South America, South Africa, Australia, New Zealand, Turkey and several European countries (Kaplan, 2002; Çirak, 2004). If we analyze the spreading situation of anthelmintic substances resistance in Europe, we can notice a slight extension of the phenomenon from west to east, affecting countries with tradition in raising horses, like England, Holland and Germany (Morariu, 2007). Thus, in some of these countries benzimidazoles resistance of cyathostomes reaches 70% or more in horse farms (Kuzmina *et Kharchenko*, 2008). Recent studies have shown the installation of benzimidazole resistance in Greece, Ukraina and Turkey (Papadoupoulos *et al.*, 2000; Çirak *et al.*, 2004; Kuzmina *et Kharchenko*, 2008).

In our country, Cernea *et al.* (2004), noted this phenomenon to horses from Bistrița Năsăud lands, where was reported cyathostomes resistance to albendazol treatment.

Due to high effectiveness and broad spectrum of action, ivermectins tend to become the most widely used class of antiparasitic substances. In Europe, as in our country, there does not exist dates showing the emergence of strongyls resistance to treatment with ivermectin (Covașă *et al.*, 2012). However, there are recent studies which showed only reduced efficacy of these compounds by reducing the period of eggs recurrence in faeces after treatment (Von Samson-Himmelstjerna *et al.*, 2007; Lyons *et al.*, 2008; Edward *et Hoffmann*, 2008; Molento *et al.*, 2008; Riccardo *et al.*, 2010; Lyons *et al.*, 2010). This was particularly highlighted for ivermectin, maintaining good efficacy for moxidectin (Lyons, 2010). It is considered that the O.P.G. from feces must be negative or very low for 8 weeks after treatment with ivermectin and 10 weeks or more in the case of moxidectin (Lyons *et al.*, 2010).

In exchange was reported in several studies, ivermectin resistance of *P. equorum*, both both in America and in Europe and Asia (Boersema *et al.*, 2002; Hearn *et Peregrine*, 2003; Kaplan *et al.*, 2006; Stoneham *et Coles*, 2006; Schougaard *et Nielsen*, 2007; Slocombe *et al.*, 2007; Von Samson-Himmelstjerna *et al.*, 2007; Lindgren *et al.*, 2008; Molento *et al.*, 2008; Sakhae *et al.*, 2011; Laugier *et al.*, 2012).

In terms of *Strogyliidae* tetrahydropyrimidines resistance, it has been described in countries such as Norway, Denmark and the US (Kaplan, 2002; Kaplan *et al.*, 2004; Von Samson-Himmelstjerna *et al.*, 2007), especially those in the cyathostomins group.

In most regions of the globe it has been reported a predominance of a small group of species (10-12) in the *Strongylidae* population of domestic horses subjected to anthelmintic treatments (Collobert-Laugier *et al.*, 2002; Osterman-Lind *et al.*, 2003; Kuzmina *et al.*, 2005;

Kuzmina *et Kharchenko*, 2008). Ten cyathostome dominant species (*C. nassatus*, *C. catinatum*, *C. calicatus*, *C. ashworti*, *C. longibursatum*, *C. goldi*, *C. pateratum*, *C. minutus*, *C. coronatus*, *C. leptostomum*) were identified as resistant to benzimidazole in various countries (Tolliver *et al.*, 1993; Lyons *et al.*, 1996; Kaplan, 2002). Beside them, the species *C. labiatus* was found resistant to benzimidazole in Olanda and Sweden (Eysker *et al.*, 1988; Osterman Lind, 2007).

Therapy failure caused use of drug combinations which originally had a high efficiency, then in most cases ascertaining the emergence of the phenomenon of multiple resistance. In this situation finding new molecules against which resistant strongyls manifest sensitivity, is an ongoing concern and urgent topical. Currently trying to find new ways to fight this phenomenon, one of the possible alternatives being phytotherapy (Cernea *et al.*, 2009).

Conclusions

1. A special emphasis in combating of resistance phenomenon, falls on screening tests of resistance, screening which must precede performing of anthelmintic treatment.
2. Benzimidazoles represent the most widely used group of antiparasitic substances until recently to combat digestive parasitosis and serous of horses, with a relatively broad spectrum. However, numerous studies have revealed a massive increase in resistance to many compounds of this class, particularly in the *Strongylidae* case.
3. If internationally numerous cases of chemoresistance have been reported, in our country this is still a weak widespread phenomenon, but ongoing.
4. We consider that low frequency of *Strongylidae* or other helminths resistance installation phenomenon to macrocyclic lactones and other antiparasitic substances has the right cause the recent use of these substances in antiparasitic treatment unlike most Western countries where they were put on the market longer.
5. The efficacy study of antiparasitic substances used in our country against strongylidosis and not only, reveals a suitable anthelmintic activity, without manifestations of resistance in the studied areas, even 100 % given as the FECRT test values.
6. We believe that the absence of resistance to ivermectin in this case are due to regular annual deworming and alternately plan in semi-intensive system, and in extensive system due to sporadic carrying out of these treatments.
7. As demonstrates in many previous studies, ivermectin remain, especially in our country, a higher class of antiparasitic compounds, both in terms of spectrum and treatment effectiveness.
8. In addition to chemical treatment of digestive parasites in horses, research in this field has expanded to other alternative means, one of which being phytotherapy , sometimes with promising results.
9. Investigations into the effectiveness of parasitosis treatments and other control measures, is a main direction in the future in equine parasitology and beyond.

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