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Determining anthelmintic-resistance of cyathostomes using anthelmintics from two drug classes

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Summary

The experiment was conducted at the "Vilniaus žirgynas" horse breeding farm in Lithuania, where horse strongyles' resistance to fenbendazole (FBZ) was detected earlier by an FECR test. The experiment included 4 naturally-infected horses. The following anthelmintics were administered to three experimental horses: first FBZ @ 7.5 mg/kg BW, and subsequently ivermectin (IVM) @ 0.2 mg/kg BW 13-14 days later. One control horse was not treated. The elimination of strongyles after treatment with FBZ lasted 35-95 hours, and 36-38 hours after treatment with IVM. In total, 87422 small strongyles (cyathostomes) were expelled in the faeces of horses treated with FBZ and IVM. The treatment with FBZ in each experimental horse reduced the strongyle faecal egg count (FEC) by 94.5%, 86.3%, and 83.1% (average 88.0%), and strongyle worm burdens by 81.4%, 67.1%, 48.1% (average 65.5%), respectively. A total of 5491 cyathostomes were collected and thirteen species of cyathostomes were found (Cylicocyclus nassatus, Cyathostomum catinatum, Cylicocyclus leptostomus, Cylicostephanus longibursatus, Cylicocyclus ashworthi, Cylicostephanus goldi, Cyathostomum pateratum, Coronocyclus labiatus, Cylicostephanus minutus, Coronocyclus labratus, Cylicocyclus insigne, Cylicostephanus calicatu, Coronocyclus coronatus). It was determined that Cylicocyclus ashworthi, Cylicocyclus nassatus, and Cylicostephanus goldi had developed strong resistance to FBZ (54.69%, 53.02%, and 34.10%, respectively); Cyathostomum catinatum was less resistant (16.83%). Cylicostephanus longibursatus and Cyathostomum pateratum were considered to be weakly resistant, whereas Cylicocyclus leptostomus exhibited no resistance at all. The results of this study indicate that the use of the two anthelmintics have a potential value for detecting anthelmintic-resistance in horse strongyles and could be used as an alternative to necropsy. However, this recommendation requires further investigation.

Keywords: horse, cyathostomes, anthelmintic-resistance

Faecal egg count reduction (FECR) today is the most frequently-used test for detection of anthelmintic--resistance. However, this test is inconclusive in estimating treatment efficacy because it does not evaluate the worm burdens. This method also has limitation because the number of helminth eggs is subject to variations during the day. When helminth eggs are absent in faeces, it is assumed that helminths have been eliminated completely from the host. Much more precise data about anthelmintic efficacy could be obtained by methods designed for evaluation of the direct effect of parasiticides on helminths. The worm burden reduction could be determined after administration of two different anthelmintics (20). For this method, horses are first treated with an anthelmintic for which parasites have been reported resistant, and then with an efficacious anthelmintic from another drug class seeking to eliminate helminths resistant to the first anthelmintic. Treatment with very efficient anthelmintics, designed to determine the species composition of a cyathostome population, have been reported (10, 13). Data from these investigations have been determined to be in agreement with necropsy surveys. Counting the helminths eliminated after treatment with two anthelmintics offers a good opportunity to determine the species composition of helminths and their resistance to the tested anthelmintic(s). Long-term investigations of this kind could help monitor any variation in the number of helminths in a population. This would allow timely identification of resistant species and contribute to the efficient control of helminths. Determining cyathostome-resistan-

ce, using anthelmintics from two drug classes, extends the scope of investigations in a small number of animals and enables examination of larger groups of animals of different ages.

The objectives of this study were: to discover the species of cyathostomes parasitizing the equids of horse breeding farm SP UAB „Vilniaus žirgynas", to evaluate the differences in efficacies after treatment with fenbendazole (FBZ) and ivermectin (IVM), thus determining resistance to FBZ, to determine the FECR correlation with the reduction of worm burden, to discuss the feasibility of the suggested methodical solution (deworming with anthelmintics from two drug classes), and to determine the advantages of this method to be used in parasitological investigations.

Material and methods

The first experiment was done to evaluate efficacy of IVM against horse strongyles. The experiment included 20 horses. Horses were divided into two groups. 10 horses (first group) were treated with IVM 0.2 mg/kg BW (Eqvalan 1.87% paste, Merial, Netherlands), other 10 horses were left untreated (control group). Fecal egg counts were determined by a modified McMaster method (8) at the day of treatment and 12 days after treatment. A sensitivity of McMaster method (8) was 20 eggs per gram of faeces.

The second experiment was conducted at the Lithuanian state-owned horse breeding farm SP UAB „Vilniaus žirgynas". The experiment included four mares of Arabian breeding (three treated and one nontreated control). The horses bore no clinical signs of disease and were naturally infected with strongyles. Their weight ranged between 500 kg to 600 kg and their ages were between 4 and 16 years old. Each of the experimental horses was kept in individual pens. Three were first treated with FBZ @ 7.5 mg/kg BW (Fenben 50% granules, Sanitas, Lithuania) and, after 14 days, were administered IVM @ 0.2 mg/kg BW (Eqvalan 1.87% paste, Merial, Netherlands). The treated horses received FBZ granules with a small amount of commercial fodder in the morning; IVM paste was spritzed under the root of the tongue. The control horse was untreated. The droppings of the four experimental horses were collected throughout the experiment and the pens were cleaned thoroughly. The droppings were collected before treatment and after drug administration, both at night and in the daytime for 3-5 days, to recover the worms passed in the faeces. Each portion of droppings was put in a polyethylene bag, weighed, and served as a source of aliquot samples for coproscopical examination and worm count. From 5 to 20% of each portion of faeces was taken as a subsample for worm speciation and prevalence. The subsamples were thoroughly chopped with a microbiological needle and pancetta and examined against a black background. All strongyles were retrieved, counted, washed with physiological solution, and fixed in 70% ethyl alcohol solution. Fecal egg counts were determined by a modified McMaster method (8) with a sensitivity of 20 eggs per gram of faeces. Each of the samples was examined thrice and an arithmetic mean was derived from the data obtained. The number of strongyles was counted using the formula A = B*100/C, where A = number of strongyles in the total portion of faeces, B = number of strongyles in the examined subsample of faeces, and C = the percent of subsample examined. Adult small strongyles (cyathostomes) recovered from faeces were cleared using 80% phenol solution in glycerin and examined morphologically under a light microscope with Nomarski differential interference contrast; magnification was 200-400 times. The species of cyathostomes were identified according to criteria described by Dvojnos and Kharchenko (6).

Anthelmintic efficacy of FBZ was evaluated in two ways: (I) the intense efficacy (IE) of FBZ, according to the FECR after deworming, was calculated using formula IE = (A – B)*100/A, where A equals the number of eggs per 1 gram of faeces before deworming and B equals the number of eggs per 1 gram of faeces after deworming, and (II) the intense efficacy of FBZ, according to the number of excreted strongyles, was evaluated using formula IE = A*100/(A + B), where A equals the number of excreted strongyles after administration of FBZ and B equals the number of excreted strongyles after administration of IVM. Resistance of cyathostomes to FBZ in the horse was determined to be present in the worm population if the drug efficacy of the FECR or worm burden was lower than 95%. A species was deemed to be highly resistant if the number of specimens recovered were 30% or more, resistant when the number of such individuals accounted for 10-20%, and weakly resistant when specimens were present at 10% or less. The experiment was conducted following the Law of the Republic of Lithuania regulating the protection and keeping of domestic animals and their use for scientific purposes.

Results and discussion

The first experiment showed that IVM was fully (100%) effective against horse strongyles. Twelve days after the treatment with IVM, coproscopical examination showed no helminth eggs in the faeces. The data of the second experiment are presented in table 1. Before treatment with FBZ, the faeces of horses con-

Tab. 1. Intense efficiency (IE) of treatment with fenbendazole (FBZ) according to the egg count per 1 g of faeces and according to the number of worms

Horse No	Egg count per 1 g of faeces		IE, %	Number of worms eliminated with faeces			IE, %
	before treatment	after treatment with FBZ		after treatment with FBZ	after treatment with IVM	total	
1	1587	87	94.5	25 327	5802	31 129	81.4
2	240	33	86.3	28 415	13 930	42 345	67.1
3	667	113	83.1	6705	7243	13 948	48.1
Control	247	347	n.c.	n.e.	n.e.	n.e.	n.c.

Explanations: n.c. – not counted, n.e. – not excreting

tained an average strongyle eggs per gram (epg) of 831. Individual epg was 240-1587. Fourteen days after treatment average epg was 78. In the faeces of individual horses' epg ranged from 33 to 113. After treatment with FBZ, strongyle eggs were found in faeces of all horses; meanwhile, on the third day after treatment with IVM, strongyle eggs were not detected. After the first deworming (with FBZ) 60.447 helminths were eliminated in the faeces (tab. 1). From 6705 to 28 415 helminths eliminated from individual horses. A total of 26 975 (5802-13 930) strongyle specimens was eliminated from horses after the treatment with IVM. The elimination of strongyles from horses treated with FBZ lasted for 35-95 hours and after the treatment with IVM it lasted 36-38 hours. Spontaneous elimination of strongyles from the intestines of the untreated control horse was not determined. The results of coproscopical examinations at the beginning and at the end of the experiment were almost identical (tab. 1).

A total of 5491 helminths was collected for identification of the species of nematodes. Identification of species revealed that all collected helminths belonged to the small strongyle (cyathostome) group. Examination of the faeces of experimental horses after deworming with FBZ and IVM showed an absence of other helminth species (*Delafondia vulgaris*, *Alfortia edentata*, *Strongylus equinus*, *Parascaris equorum* or *Oxyuris equi*). It was determined that the examined horse-breeding farm was infected with 13 species of cyathostomes (tab. 2). To summarize the result of the species identification, it was determined that *Cylicocyclus ashworthi*, *Cylicocyclus nassatus*, and *Cylicostephanus goldi* had developed strong resistance to FBZ (54.69%, 53.02%, and 34.10% respectively); *Cyathostomum catinatum* was less resistant (16.83%). The weakly-resistant species were *Cylicostephanus longibursatus* and *Cyathostomum pateratum*; whereas, *Cylicocyclus leptostomus* showed no resistance at all. The presence of some species was in such small numbers that no basis could be determined with reference to resistance.

There has been very little necessity in Lithuania for extended investigations of cyathostomes in horses in recent years because of the high performance of the benzimidazoles. Unfortunately, there already have been reports of anthelmintic-resistance of cyathostomes on some horse breeding farms (19).

A total of 5491 cyathostome specimens was collected on the farm in the current study for determining the species composition of the helminth population. It was determined that the horses were infected with only cyathostomes. According to literary sources, this group of helminths represents the greater part of a parasite population parasitizing the horse (2).

A total of 13 species of cyathostomes has been identified. This number may vary depending on the extent of the investigations. When experiments are based on 10-20 horses, the number of identified cyathostomes amounts to about 15 (9, 13, 17). Taking into account the scope of our investigations, the obtained results do not contradict the mentioned indices of the number of cyathostome species. The results correspond with the observation of other authors, that each horse is invaded usually by a multi-variety population of cyathostomes (3, 13, 17), although only about 6-10 species account for the major portion of the population (3, 12--14). *Cylicocyclus nassatus* (42.18%), *Cyathostomum catinatum* (22.73%), *Cylicocyclus leptostomus* (13.60%) were the most abundant species in the investigated cases (tab. 2). According to the literature, *Cylicocyclus nassatus*, *Cyathostomum catinatum*, *Cylicocyclus leptostomus*, and *Cylicostephanus longibursatus* species are rather widespread in different geographical regions (2-4, 7, 10, 15). The species of cyathostomes resistant to anthelmintics usually are abundant and widespread. Bauer (1), in his report, mentions 13 species of cyathostomes resistant to anthelmintics. Ten of the mentioned species account for more than 95% of a small strongyle population in most horses. Moreover, according to literary references, these same species of cyathostomes develop resistance to benzimidazoles in different countries. Resistance of *Cylicocyclus nassatus*, *Cylicostephanus goldi*, *Cylicostephanus longibursatus*, *Cylicostephanus calicatus*, *Coronocyclus labiatus*, and *Coronocyclus coronatus* to benzimidazoles has been reported in the USA and in Europe (5, 16, 17). The results of our investigation are in accordance with studies of other authors. *Cylicocyclus nassatus* was the most abundant variety with the highest degree of resistance (53.02%). However the abundance of a species did not always reflect the degree of resistance to drugs.

Tab. 2. Cyathostome species parasitizing the equids of the horse breeding farm SP UAB „Vilniaus žirgynas"

Species	Found		Abundance %	Resistant %
	from-to	total		
Cylicocyclus nassatus	143-1601	2316	42.18	53.02
Cylicocyclus leptostomus	1-744	747	13.60	0
Cylicocyclus ashworthi	1-424	426	7.76	54.69
Cylicocyclus insigne	0-2	2	0.04	n.d.
Cylicostephanus longibursatus	1-222	428	7.79	7.71
Cylicostephanus goldi	48-70	173	3.15	34.10
Cylicostephanus minutus	0-6	7	0.13	n.d.
Cylicostephanus calicatus	0-1	1	0.02	n.d.
Coronocyclus labiatus	0-19	19	0.35	n.d.
Coronocyclus labratus	0-3	4	0.07	n.d.
Coronocyclus coronatus	0-1	1	0.02	n.d.
Cyathostomum catinatum	70-1009	1248	22.73	16.83
Cyathostomum pateratum	0-119	119	2.17	0.84

The resistance of the second most abundant variety, *Cyathostomum catinatum*, was only 16.83%. It must be pointed out that the third most abundant species, *Cylicocyclus leptostomus*, was absolutely susceptible to FBZ.

After treatment with FBZ, the time of elimination depended on the intensity of cyathostome invasion and less on the proportion of resistant individuals in the population. When the invasion was strongest, the elimination of cyathostomes took longer than in the case of weaker invasion. Elimination of cyathostomes after deworming with IVM was considerably shorter. These data are in correlation with the established differences of the time of elimination of strongyles after treatment with different drugs (13). The variations of helminth elimination dynamics can be explained by different mode of action of drugs (11).

To summarize, we can say that the FECR method is most frequently applied in investigations of anthelmintic resistance of strongyles. This test is based on evaluation of faecal egg count before and after treatment without estimating the effect of treatment on worm burdens. This method is not very precise because the number of eggs in faeces does not reflect the actual worm burden in the intestines and may vary during a day (13). The number of detected epg of the three experimental horses, before treatment with FBZ, was 1587, 240, and 667, respectively (tab. 1). Unfortunately, the coproscopical investigations did not reflect the actual intensity of invasion. After deworming with two anthelmintics, the number of eliminated worms from the treated horses was 31 129, 42 345 and 13 948, respectively. Thus, the FECR method is based on a low sensitivity phenotypical resistance parameter. Therefore, it is necessary to seek other methods or evaluation criteria which would facilitate more precise and rapid identification of anthelmintic-resistance. The importance of extense efficiency (EE), showing the number of animals that contained no worm burden and the faeces contained no helminth eggs after deworming, has been discussed (18, 19). Though this index provides important information about the tolerance of helminths to drugs, its quantification is also based on the calculation of epg in faeces. Combined application of anthelmintics from two classes of drugs would provide more accurate information about resistance to anthelmintic chemical compounds. The horses were preliminary treated with anthelmintic to which cyatostomes are reported to have developed tolerance. The resistant cyathostomes then are collected with the horse faeces. Later horses are treated with a high performance anthelmintic, which has not been used for deworming before. In our case, ivermectin was such anthelmintic. Examination of smaller amounts of faeces allows precise evaluation of the actual composition of the population and the portion of resistant cyatostomes in it. Based on the results of more detailed analysis this methodical solution would allow determining the tolerance of cyatostomes to anthelmintics without slaughtering the animals. Moreover, this would be a direct evaluation of the efficiency of deworming and cyatostome resistance. In our opinion, only more detailed further investigations will allow final evaluation of the obtained preliminary results.

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