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Efficacy of two Anthelmintic Products on Strongyles in Horses from Stud Farms in Romania

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Abstract

Nematodes belonging to the group of equine strongyles have a huge impact on equine health. Studies in horse establishments worldwide have shown a tremendous occurrence of these parasites under largely different geographic and climatic conditions. Accordingly, decades of strongyle control have been based on regular and frequent preventive treatments with anthelmintics and, subsequently, this has led to increasing levels of anthelmintic resistance. Therefore, it is essential that investigations into the efficacy of current anthelmintics in different locations are performed to help future reliable control programmes. This paper reports preliminary data of a survey conducted in Romania to evaluate the efficacy of two commonly used anthelmintics (fenbendazole - FBZ and ivermectin - IVM) against horse strongyles, using a faecal egg count reduction test (FECRT). Horses with a strongyle faecal egg count (FEC) of ≥50 eggs per gram (EPG) were enrolled into the study. The anthelmintic efficacy was determined by calculating the percentage of reduction in FEC between the group mean at Day 0 and Days 14 post-treatment. Individual strongyle faecal egg count reduction tests (n=137) were performed on three stud farms (R, S, C) in which 108 and 29 horses were administered ivermectin and fenbendazole, respectively. The results of the study have shown that the efficacy was within the expected range with no signs of anthelmintic resistance for IVM (98.2-100%). However, FBZ resistance was suspected in one stud (the group mean FECR of 75.4%). Thus, further studies are planned to investigate extension of the anthelmintic resistance in horse parasites including an increased number and different horse establishments in Romania, in order to design realistic control programmes carrying a limited risk of selection for anthelmintic resistance and improve overall health of the horses.

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Keywords: anthelmintic drugs, efficacy, strongyles, horses, Romania

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1. Introduction

Nematodes (Nematoda: Strongylidae) belonging to the group of equine strongyles have a huge impact on equine health. Studies in horse establishments world-wide have shown a tremendous occurrence of these parasites under largely different geographic and climatic conditions (Lyons et al., 1999). Wherever horses graze, there are strongyle nematodes (Love et al., 1999). Their clinical importance is well-documented, as infection can lead to weight-loss and unthriftiness, disease symptoms dominated by colic and diarrhea, and death in severe cases (Drudge and Lyons, 1986; Reinemeyer, 1986). Accordingly, decades of strongyle control have been based on regular and frequent preventive treatments with anthelmintics (dewormers). However, this has led to increasing levels of anthelmintic resistance**,** now affecting a majority of helminth species and virtually all products on the equine market (Kaplan, 2002, 2004). For the treatment and control of nematode infections in horses, three major anthelmintic classes are available: the benzimidazoles (BZs), the tetrahydropyrimidine- pyrantel (PYR) and the macrocyclic lactones (MLs). Traditional control strategies mainly rely on the strategic application of anthelmintics.

The great dilemma in controlling internal parasites in horses and other domestic animals is that, historically, after a period of usage of antiparasitic drugs, resistance of some species occurred (Uhlinger, 1991). Therefore, following decades of routine and frequent anthelmintic applications, many cyathostomin populations on horse farms in industrialized countries are considered as resistant to BZ anthelmintics. The anthelmintic resistance has become a major problem in the veterinary field during recent years and threatens both agricultural income and animal welfare. In horses, benzimidazole resistance is widespread among the cyathostomins and the recent finding of apparent drug-resistance in ivermectin and moxidectin of ascarids is alarming (Wolstenholme et al., 2004). Reports of anthelmintic resistance in equine strongyles are increasing in managed horses world-wide (Kaplan et al., 2004). Reports from the US (Lyons et al., 2008) and Germany (von Samson-Himmelstjerna et al., 2007) revealed a shortened strongyle egg reappearance period (ERP) after ivermectin treatment, which is being interpreted as early indications of developing resistance. Thus, on horse farms the efficacy of anthelmintic treatments should be examined routinely for each drug class. Moreover, it is essential that investigations into the efficacy of current anthelmintics in different locations are performed to help inform control programmes (Nielsen, 2012; Lester et al., 2013).

Data on anthelmintic efficacy in Romanian horses are scarce and fragmentary. Therefore, the aim of the present study was to conducte a preliminary survey to evaluate the efficacy of two commonly used anthelmintics (fenbendazole - FBZ and ivermectin - IVM) against horse strongyles in Romania.

2. Materials and Methods

Farms and animals

The study was carried out between March 2013 and September 2014 on three stud farms from North-Eastern (R), Center (S) and South-Eastern (C) Romania. The type of the stud farms selected for the study included breeding and training. The selection criteria of the farms consisted of willingness of the farm manager to cooperate, presence of at least 25 animals in the farm, and the horses had received no anthelmintic treatment for at least 10 weeks prior to testing. Two farms (R, S) regularly used anthelmintics from different classes through the year, up to three treatments per year. The last farm (C) irregularly used anthelmintics from different classes, more usually benzimidazoles.

Study design and *Faecal analysis*

Naturally infected horses from three stud farms (R, S, C) were enrolled in a study to test two commercially available anthelmintic products. A faecal screening test was performed in 195 horses. Only animals (n=137) with values of $\geq$50 EPG were included in the study. The age of the horses ranged from 1 to 21 years with a mean of 6.3 years. Of them, 108 and 29 horses were administered ivermectin and fenbendazole, respectively. The horses were weighed, given the manufacturer's recommended dose of the product orally (Day 0), and individual faecal samples were collected on Day 0 and Day 14 post-treatment, to perform the *Faecal Egg Count Reduction Test* (FECRT) as described (Coles et al., 1992). In Table 1 is presented the detailed treatment design carried-out at day 0.

Faecal samples were analysed quantitatively, using a modified McMaster technique with a sensitivity of 25 eggs per gram (EPG).

Data analysis - Faecal Egg Count Reduction Test (FECRT)

Percentage reduction in FEC were determined for each horse, with the following formula [(pre-treatment EPG - post-treatment EPG)/pre-treatment EPG] x 100. Mean values for percentage efficacy were calculated for each treatment group on each farm.

The anthelmintic efficacy was determined by calculating the percentage of reduction in FEC between the group mean at Day 0 and Days 14 post-treatment. Treatments were categorized as effective for FECR ≥90 for FBZ and ≥95% for IVM, respectively, equivocal for FECR from 80 to 90%, and ineffective for FECR ≤80% (Kaplan, 2004).

3. Results and Discussion

All together, 195 horses were investigated within the screening procedure and an FECRT was performed on 137 horses. The EPG value of ≥50 was used as a cut off for inclusion in the study to evaluate the efficacy of two commonly used anthelmintics, IVM (n=108) and FBZ (n=29) against strongyles in horses from stud farms in Romania. Mean percentage efficacy of the IVM and FBZ in each farm surveyed is reported in the Table 1 together with the mean EPG value pre- and post-treatment for each horse group. The frequency distribution of individual faecal egg counts is depicted in Figure 1.

Fourteen days after treatment, IVM was evaluated to be fully susceptible in all three farms examined, with mean FECRs of 100% in two farms and 98.2% in one farm, respectively. FBZ resistance was suspected in the farm where the drug was tested, with the mean FECR registered of 75.4%.

Table 1. The efficacy of two anthelmintics evaluated against strongyles in three horse stud farms in Romania
[group arithmetic mean faecal egg count (mean EPG); EPG-range; standard deviation (SD), efficacy of the treatments (FECR, in %)]

Drug	No of horses treated / screened	EPG-counts						FECR (%)
		Pre-treatment			Post-treatment			
		Mean EPG	SD	EPG range	Mean EPG	SD	EPG range	
Ivermectine (IVM) (0.2 mg/kg)								
Farm R	54 / 70	736	633.765	50-2225	0	0	0	*100*
Farm S	34 / 55	911	738.9418	50-1975	9.7	24.90792	0-100	*98.9*
Farm C	20 / 35	876	1040.953	50-3850	16.3	13.33	0-125	*98.2*
Fenbendazole (FBZ) (7.5 mg/kg)								
Farm C	29/35	884	961.2216	50-4000	217	380.5015	0 - 1500	*75.4*

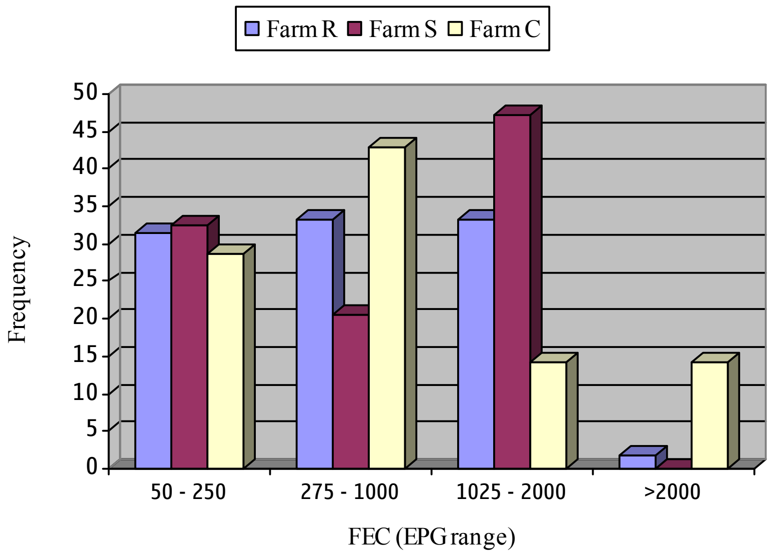

Figure 1. Frequency distribution of strongyle faecal eggs counts (FECs) before treatment.
Faecal samples were obtained from 137 horses in three stud farms in Romania

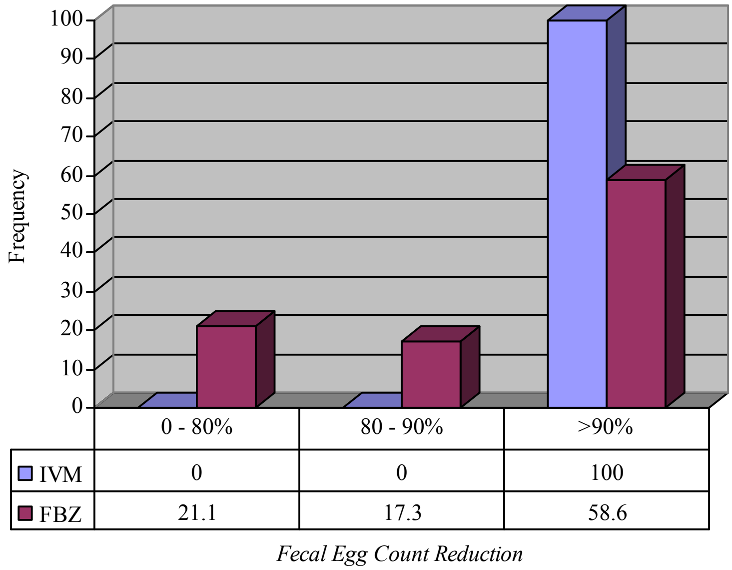

Figure 2. Frequency distribution of individual percentage (%) of the efficacy of ivermectin and fenbendazole against strongyles in horses from stud farms in Romania

This study has shown that strongyle infections are highly prevalent in horses residing in stud farms, with high infection intensities according to an over dispersed distribution pattern (Fig. 1). Overall, 70% of the horses exceeded the value of 250 strongyle EPG, which is considered the cut-off within the new equine strongyle control strategy based on targeted selective treatment. One of the basic principles of selective anthelmintic treatment is a consistency of the relative magnitude of strongyle FECs of individual horses over time (Duncan and Love, 1991). Therefore, identification of high egg shedders within the herd is an essential goal (Gomez and Georgi, 1991; Nielsen et al., 2006; Eysker et al., 2008). It is proposed that by identifying animals regarded as 'high egg shedders', this will enable farms and studs to implement more targeted treatment approaches to helminth control (Nielsen et al., 2006).

There is known that acquisition of information on natural distribution patterns will help in establishing appropriate faecal worm egg count (FWEC) thresholds at which horses should be treated with an anthelmintic product (commonly quoted as 200 - 250 EPG (Uhlinger, 1993; Kaplan and Nielsen, 2010).

Previous studies in Romania (Buzatu et al., 2013, 2014) have indicated that 86.23% and 69.01% of horses residing in studs and working horses, respectively, were positive for strongyle. Of them, 72.5% of horses from studs and 44.44% of the working horses exceeded the cut-off value of 250 strongyle EPG. Therefore, the results of the present study are in line with these data, confirming a higher parasitic infection pressure within the horse populations raised in stud farms. This higher parasitic infection pressure was expected since these horses use the same pastures. It is well known that removing feces from pasture would lower the infection pressure enormously but probably needs to be done every week to keep the pasture sufficiently safe (Herd, 1986, 1990).

Although strongyle parasites are generally regarded as ubiquitous, egg count levels were markedly higher than usually reported from well managed horse populations in developed countries. For instance, in a recently published German study, 2000 horses were found to have faecal egg counts primarily within the 0–500 EPG range (Fritzen et al., 2010). However, our findings are in line with similar studies in Romania (Cernea et al., 2003; Ionita et al., 2013) and Italy (Traversa et al., 2007) where high intensive strongyle infections have been reported.

With regards to the anthelmintic resistance, in the USA, the anthelmintic resistant small strongyles are more widespread than elsewhere and this might be due to the fact that horses usually receive antiparasitic drugs four or more times per year (Kaplan, 2002; Kaplan et al., 2004).

When referring to Europe, the presence of resistance in Switzerland was reported in more than the half of examined herds (Meier and Hertzberg, 2005) or in the vast majority/totality of surveyed farms in Sweden (Nilsson et al., 1989), Denmark (Bjorn et al., 1991), England (Fisher et al., 1992), Slovak Republic (Varady et al., 2000) or

Germany (Wirtherle et al., 2004). In Itlay, the prevalence of anthelmintics resistant cyathostomes appears to be less than that described in other European countries. However, a recent study has indicates that multiple drug resistant equine cyathostomes are present in the central and southern regions of Italy: resistance to fenbendazole was declared on six farms (37.5%) and suspected in two farms (12.5%), with FECR values ranging from 41% to 88.3%, while resistance to pyrantel was found in two farms (12.5%) and was suspected in one case (6.2%), with FECR values ranging from 43% to 85.4%. Macrocyclic lactones remained effective on all farms (Traversa et al., 2007).

In our study, the anthelmintic efficacy was within the expected range, with no signs of anthelmintic resistance for ivermectine, in all three stud farms surveyed. As expected, the mean percentage efficacy of IVM in eliminating strongyle eggs from animals' feces ranged from 98% to 100% for IVM in all stud farms. It should be mentioned that in all surveyed farms the frequency of the anthelmintic treatment was usually lower than four per year, which explain the conserved efficacy of the drug.

However, FBZ resistance was suspected in one stud, as about 40% of the treated horses presented individual percentage of the efficacy bellow the cut-off of 90%. Of them, for 21.1% of the tested horses use of FBZ was virtually ineffective, while for 20% was equivocal.

The suspected resistance to FBZ registered herein is of interes since anthelmintic benzimidazoles resistance, including, for fenbendazole, thiabendazole, mebendazole, and albendazole, has been previously reported only in few studies in north-western Romania (Cernea et al., 2005). Thus, these findings require further geographical and numerically extended investigation of farms, to assess the real situation of the anthelmintic phenomenon in equine strongyle populations in Romania.

4. Conclusions

The results of the present survey showed that strongyle infections are highly prevalent in stud farms in Romania and clearly demonstrated the anthelmintic efficacy of ivermectine. However, FBZ resistance was suspected in one stud. Thus, further studies are planned to investigate extension of this phenomenon in horse parasites including an increased number and different horse establishments in Romania. Moreover, the findings are expected to serve as a reference for future investigations and control actions, in order to design realistic control programmes carrying a limited risk of selection for anthelmintic resistance and improve overall health of the horses.

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