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Anthelmintics efficacy against intestinal strongyles in horses of Sardinia, Italy

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ABSTRACT

Intestinal strongyles (IS) are the most important parasites of equids, due to their high prevalence worldwide, pathogenicity and the spread of drug-resistant populations. Despite the large number of horses bred in Sardinia Island, Italy, no data are available on the efficacy of anthelmintic compounds in the control of horse strongylosis. Therefore the aim of the present study was to evaluate the efficacy of five commercial anthelmintic formulations containing fenbendazole (FBZ), pyrantel (PYR), moxidectin (MOX) and two ivermectin formulations (IVM1 and IVM2) against IS in Sardinia by performing a fecal egg count reduction test (FECRT) and investigating the egg reappearance period (ERP) after treatment. In total, 74 horses from 7 farms were examined. Coprocultures performed for individual fecal samples collected at the day of the treatment revealed that cyathostomins were the predominant parasitic species (98.6%). The FECR for all horses belonging to the treatment groups after two weeks was ≥95% with a 95% C.I. >90%. The expected ERP did not decrease in any of the treatment group as FECR values <90% were found at D60 for FBZ, at D90 for PYR and IVM1, at D150 for IVM2. All horses treated with MOX showed FECRT >90% for the entire duration of the trial until D150. The results of the present survey indicate that drug-resistant cyathostomin populations are not present in the examined horse population, contrariwise to what observed in other Italian and European regions. The reasons and implications of these results are discussed.

1. Introduction

The most common and pathogenic parasitic nematodes of horses are members of the family Strongylidae, which includes large (Strongylinae Subfamily) and small (Cyathostominae Subfamily) strongyles. Intestinal strongyles (IS) of horses have a primary importance in equine medicine, for their worldwide distribution and impact on health and performance of infected animals (Heidi and Wade, 2009). In particular, small strongyles, also known as cyathostomins, are regarded as the most important helminth parasites of horses for their cosmopolitan diffusion and their pathogenic potential at both larval and adult stages (Peregrine et al., 2014). Currently, the control of IS infections is based on the use of systematic anthelmintic treatments and, when applied, on management measures, e.g. removal of feces from paddocks and grazing rotation (von Samson-Himmelstjerna, 2012; Nielsen et al., 2014; Papini et al., 2015). The abuse of anthelmintic for a long time in the so-called "blanket treatments" approach

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has caused a worldwide distribution of anthelmintic resistance (AR) in cyathostomin populations (Heidi and Wade, 2009; von Samson-Himmelstjerna, 2012; Nielsen et al., 2014). In particular, resistance to BZ such as fenbendazole (FBZ) is common and widespread, and increasingly reported for tetrahydropyrimidines, such as pyrantel salts (PYR). Macrocyclic lactones (MLs), i.e. ivermectin (IVM) and moxidectin (MOX), are still the most effective compounds against cyathostomins, despite reduced efficacy has been reported in both Europe and the Americas, especially for IVM (Traversa et al., 2009; Lyons et al., 2011; Molento et al., 2012; Traversa et al., 2012). In Italy, cyathostomins are widespread in horse populations, with prevalence up to 100% (Traldi et al., 1988; Piergili-Fioretti et al., 2005; Pilo et al., 2012); resistance to BZ, PYR and IVM has been reported in Italy (Traversa et al., 2009; Genchi et al., 1992; Traversa et al., 2007), with reduction of the Egg Reappearance Period (ERP) of IVM and MOX in few premises (Geurden et al., 2014). The evaluation of the ERP is crucial in understanding a possible onset of reduced efficacy in these parasiticides, especially for macrocyclic lactones (Sangster, 1999). It is worthy of note that the treatment efficacy in most studies is evaluated through a fecal egg count reduction test (FECRT) at up to 21 days after treatment. This approach might underestimate a potential reduction in anthelmintic efficacy of MLs, as reduced activity of IVM and MOX is initially apparent from a shortened ERP after treatment, and not necessarily from a reduced efficacy within few weeks after treatment (Lyons et al., 2011).

Despite the large number of horses bred in Sardinia island of Italy (17,066 heads, representing 4.3% of the national heritage 395,913), no reliable data are available on the efficacy of anthelmintics in this area (Montinaro et al., 2002). Therefore, the present study aimed at updating current knowledge on the efficacy and ERP of five commercial anthelmintic formulations commonly used for the control of IS in horses bred in Sardinia.

2. Materials and methods

A total of 74 horses, 40 females and 34 males ranging from 6 months to 6 years old from 7 premises in Sardinia were examined in 2010 for the presence of IS infection. All the tested animals were born and bred in Sardinia.

No anthelmintic treatments were performed in selected animals in the three months before the trial. In these farms breeders usually performed anthelmintic treatments once a year in the horses kept grazing and in paddock, while every 6 months in those animals living in boxes. All horses enrolled in the trial were kept under the same original farming conditions until the end of the study. Five different anthelmintics (oral paste/gel) licensed in Italy were evaluated; these were the most used in breedings in Sardinia: FBZ [Panacur® pasta, MSD], PYR [Strike®, os pasta Acme], two containing ivermectin (IVM1) [Eqvalan® os pasta Merial], (IVM2) [Eraquell® os pasta Virbac] and MOX [Equest® os gel Zoetis]. Animals were selected and randomly enrolled in one of the five treatment groups according to the number of horses present in each farm as shown in Table 1. Treatment was administered per os on Day (D) 0 after the estimation of the weight of each horse using a girth tape (Pavo and Virbac Horse Weight Tape®). All horses were observed for at least one hour after treatment to ensure complete swallowing of the drug and to record any side effects (e.g., sweating, mydriasis, colics). All the treatments were administered by the same person and laboratory personnel did not know to which group the sample belonged. The individual FECs of each horse was determined at day-7 prior to the FECRT, and then weekly in the first month after treatment (i.e., D7, D14, D21, D28), and thereafter, biweekly for the following 4 months (i.e., D45, D60, D75, D90, D105, D120, D135, D150) using McMaster slides according to Raynaud (Raynaud, 1970). The FECRT was carried out according to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for detection of anthelmintic resistance in horses (Coles et al., 1992). Percentage reductions in the fecal egg count were determined for each treatment group with the following formula: 100 × (1 − post-treatment EPG arithmetic mean/pre-treatment EPG arithmetic mean).

The cut-off limits for establishing appropriate efficacy were arithmetic means FECR of 95% for MLs and >90% for FBZ and PYR (Kaplan and Nielsen, 2010) and a 95% confidence interval (CI) of efficacy was calculated. The results of the FECRs were interpreted for all the compounds as follows: (1) resistance present if FECR <90% for FBZ and PYR, ≥95% for MLs and the lower 95% confidence limit (LCL) <90%, (2) resistance suspected if FECR ≥90% for FBZ and PYR, ≥95% for MLs and/or LCL <90% and (3) no resistance if FECR ≥90% for FBZ and PYR, ≥95% for MLs and LCL >90%.

The evaluation of the egg reappearance period (ERP) was also assessed, being defined as the time between treatment and the first breaching of a 90% efficacy threshold (Geurden et al., 2014) and based on the group arithmetic mean FEC. The standard minimum ERP used were 42 days for PYR, 42–56 days for FBZ, 56–70 days for IVM and >91 days for MOX (Stratford et al., 2011).

Coprocultures were performed at D0 for each fecal sample and up to D150 in horses with EPG ≥ 150, in order to allow the in vitro growth of third stage strongyle larvae (L3) (Roberts and O'Sullivan, 1950), that were thereafter identified using the morphological keys by MAFF (MAFF (Ministry of Agriculture Fisheries and Food), 1986).

Table 1
Anthelmintics evaluated for efficacy against horse strongyles in a population of horses from Sardinia Island, Italy.

Group	Horses enrolled	Commercial name	Company	Molecule	Administration	Dose
IVM1	15	Eqvalan®	Merial	Ivermectin	Oral	0.2 mg/kg BW
IVM2	17	Eraquell®	Virbac	Ivermectin	Oral	0.2 mg/kg BW
MOX	10	Equest®	Zoetis	Moxidectin	Oral	0.4 mg/kg BW
PYR	19	Strike®	Acme	Pyrantel pamoate	Oral	6.6 mg/kg BW
FBZ	13	Panacur®	MSD	Fenbendazole	Oral	7.5 mg/kg BW