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Anthelmintic efficacy against cyathostomins in horses in Southern England

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ABSTRACT

Cyathostomins are considered to be the most important group of helminths to affect equids due to their high prevalence, potential pathogenicity and ability to develop anthelmintic resistance. Their control relies almost exclusively on frequent anthelmintic use. Currently, fenbendazole (FBZ), pyrantel embonate (PYR), ivermectin (IVM) and moxidectin (MOX) are licensed for use in horses in the UK. With no new anthelmintics likely to be licensed in the near future, it is essential that investigations into the efficacy of current anthelmintics in different locations are performed to help inform control programmes. Here, efficacy of FBZ, PYR, IVM and MOX in horse populations in the South of England was investigated. Horses with a strongyle faecal egg count (FEC) of ≥50 eggs per gram (EPG) were enrolled onto a faecal egg count reduction test (FECRT) study. Efficacy was determined by calculating the percentage reduction in FEC between the group mean at Day 0 and 14 days post-treatment. Efficacy was indicated when a group arithmetic faecal egg count reduction (FECR) of ≥90% was recorded for FBZ and PYR, and ≥95% for IVM and MOX. Between March and December 2012, 404 FECRT were performed on 12 yards examining 101, 110, 93 and 100 equids for FBZ, PYR, IVM, and MOX, respectively. FBZ resistance was identified on all yards (mean FECR range 0–65.8%). On 10 of 12 yards, PYR efficacy was >90% (91.0–99.4%) and on two yards, PYR resistance was suspected (86.8–87.2%). IVM (96.4–100%) and MOX (99.9–100%) were >95% efficacious on all yards. As the prevalence of FBZ resistance was 100%, the future use of this anthelmintic for the control of strongyles should be questioned. PYR should be used strategically to reduce reliance on the macrocyclic lactone class products. Over-dispersion of FEC between horses was observed (average $k = 0.21$) with 80% of the strongyle eggs counted measured in 15% of horses tested, strongly supporting the application of targeted helminth control programmes in this host species.

1. Introduction

Cyathostomins are considered to be the most important group of helminths that affect equids; this is due to their prevalence, potential pathogenicity and ability to develop

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anthelmintic resistance. Virtually all grazing horses are at risk of infection and as such, appropriate control measures are necessary. Most infections are well tolerated; however the most serious consequence of infection is the mass emergence of encysted cyathostomin larvae from the large intestinal wall, which can result in larval cyathostominosis, a colitis characterized by diarrhoea, rapid weight loss and ventral oedema, which can be fatal in up to 50% of cases (Love et al., 1999).

Anthelmintics comprise the mainstay of parasite control. Three classes of broad-spectrum anthelmintics are licensed for use against adult cyathostomins in the UK. The anthelmintics currently available are: fenbendazole (FBZ), a benzimidazole (BZD), pyrantel embonate (PYR), a tetrahydropyrimidine (THP), and the macrocyclic lactones (MLs), ivermectin (IVM) and moxidectin (MOX). Long term frequent use of anthelmintics has contributed to the development and spread of anthelmintic resistance (Kaplan, 2002, 2004; Matthews, 2008): BZD resistance in cyathostomins is widespread, (Kaplan et al., 2004; Osterman Lind et al., 2007; Traversa et al., 2012) and reduced sensitivity of cyathostomins to pyrantel salts is very common in some geographic locales (Kaplan et al., 2004; Comer et al., 2006; Lind et al., 2007; Traversa et al., 2007). In addition, single cyathostomin populations have been identified exhibiting both FBZ and PYR resistance (Kaplan, 2004; Traversa et al., 2007). A reduction in egg reappearance period (ERP) is thought to be an early indicator of resistance. A reduction in strongyle ERP from over 8 weeks (Borgsteede et al., 1993) down to 4 weeks post IVM administration has been described (von Samson-Himmelstjerna et al., 2007; Lyons et al., 2008; Molento et al., 2008) and a shortened strongyle ERP after MOX administration has been reported in Kentucky (Rossano et al., 2010). Concurrent reduced efficacy of all three broad spectrum anthelmintic classes has also been identified in Brazil (Molento et al., 2008; Canever et al., 2013).

There are no new anthelmintic classes likely to be licensed for use in horses in the short to medium term, so it is essential to assess the efficacy of the currently available products to make informed decisions for treatment and control. The primary aim of control regimens should focus on the preservation of anthelmintic-sensitive nematode populations, which needs to be balanced with minimizing the risk of parasite-associated disease. The aim of this study was to determine efficacy of all three classes of anthelmintic against strongyle infections in horses based on yards across Southern England.

2. Materials and methods

2.1. Study population

Boarding stables that were British Horse Society (BHS) approved were approached using the BHS website, contacted via email and asked to participate. In addition, boarding stables, competition and welfare yards that were clients of the Bell Equine Veterinary Clinic and House and Jackson Veterinary Clinic were recruited onto the study. All horses had access to grazing and had been treated with an ML within the last 6 months. Each yard was supplied with a questionnaire to complete, which provided information on the yard (yard type, acreage, number of horses), anthelmintic usage (frequency, last product used, type of worming programme) and management practices.

2.2. Sample collection, and FEC methodology

Fresh faecal samples were collected from all horses resident on each yard. Pre-labelled zip-lock bags were provided for sample collection, identification and submission of samples. On the first sampling occasion, the pre-labelled bags were left blank for the horse owners to write the name and age of each horse on each bag. On subsequent sampling occasions, pre-labelled bags with the horse's names were supplied. Horse owners were asked to collect at least three faecal boluses from a freshly voided motion and to place these into a zip-lock bag, expelling all the air before sealing. The samples were sent immediately to Moredun Research Institute (MRI) and stored at approximately 4 °C. All samples were processed within 4 days of collection. Prior to processing, all samples were logged onto data capture forms. The date of sample arrival, the date of processing, each horse's name and age were all recorded. Samples were recorded in batches according to which yards they came from. A modification of the salt flotation method (Christie and Jackson, 1982), sensitive down to 1 egg per gram (EPG) was used (Bartley and Elsheika, 2011). All FEC were performed in duplicate and an average of the two counts taken to estimate EPG for each sample. On 11 of the 16 yards recruited, positive FEC samples from the first screening occasion were pooled and the eggs cultured to third stage larvae. In brief, faecal samples from individual horses were formed into fist-sized balls and placed into a 500 ml container lined with a polythene bag. A further polythene bag was used to cover the container, which was pierced several times before incubation at 22 °C. After 14 days, the container was removed and flooded with tepid water and allowed to stand for four hours. The contents of the container were poured over a filter made from two layers of filter paper, the filter was then placed on top of a 200 ml jar filled with tepid water, so that the filter paper was flush with the water and left overnight so that any larvae could migrate through the filter paper into the jar. The filter was removed and the contents of the jar were siphoned off until approximately 2 cm of liquid remained. This was then poured into a flask, where it was stored at approximately 4 °C until the sample was counted. Third-stage larvae were identified according to MAFF (1986). On three yards, the FEC were too low for culture to be performed and on another two yards, eggs were not cultured to larvae due to time constraints.

2.3. Faecal egg count reduction tests (FECRT)

Faecal samples were obtained from all equids at each site once the minimum ERP of the previously administered anthelmintic had passed, the standard minimum ERP used were 6 weeks for FBZ and PYR, 8 weeks for IVM and 13 weeks for MOX (Stratford et al., 2011). Horses with strongyle FEC of $\geq$50 EPG were included and administered *per os* with anthelmintic on Day 0 at the following