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Anthelmintic efficacy on UK Thoroughbred stud farms

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ABSTRACT

Anthelmintic drugs have been applied indiscriminately to control horse nematodes for over 40 years. We undertook a comprehensive study to investigate efficacy of the four available broad-spectrum anthelmintic drugs on 16 Thoroughbred stud farms using the faecal egg count reduction test. Efficacy against strongyles was determined by calculating the percentage of reduction in faecal egg count between the group mean at Day 0 and Days 14–17 post-treatment and the 95% lower confidence intervals estimated by non-parametric bootstrapping. Individual strongyle faecal egg count reduction tests ($n = 429$) were performed in which 179, 131, 89 and 30 horses were administered ivermectin, moxidectin, pyrantel and fenbendazole, respectively. Moxidectin was efficacious in all tests (faecal egg count reduction range: 99.8–100%; 95% lower confidence intervals range: 96.8–100%) and reduced efficacy of ivermectin (faecal egg count reduction range: 85.7–100%; 95% lower confidence intervals range: 65–100%) was observed in one group of yearlings. Reduced pyrantel efficacy was observed in five groups of yearlings (faecal egg count reduction range: 0–73%; 95% lower confidence intervals range: 0–59.5%), but pyrantel was found to be efficacious when administered to mares (faecal egg count reduction range: 98–99.4%; 95% lower confidence intervals range: 91.8–99.3%). Low efficacy of fenbendazole was always observed (faecal egg count reduction range: 0.4–41%; 95% lower confidence intervals not calculable). Two further methods for estimating efficacy were applied and outputs obtained using all methodologies were in agreement. Efficacy against *Parascaris equorum* was assessed on four farms: fenbendazole had acceptable efficacy (faecal egg count reduction range: 97.5–99.9%; 95% lower confidence intervals range: 96.3–99.1%), but reduced efficacy of ivermectin was observed (faecal egg count reduction range: 25.5–91.2%; 95% lower confidence intervals range: 6.7–82.4%). Strongyle faecal egg count were analysed at approximately 2 week intervals for up to 12 weeks after anthelmintic drug administration to determine the egg reappearance period for moxidectin, ivermectin and pyrantel. The egg reappearance period for all three anthelmintic drugs was shorter than previously observed. Overall, our results indicate that ivermectin and moxidectin administration provided acceptable efficacy at 14 days; however, egg reappearance period results suggest that these products are working less effectively than measured previously. As shortened egg reappearance period is believed to be an early indicator of resistance, this highlights the issue of impending multi-drug resistance in strongyles on stud farms.

1. Introduction

Frequent, all-encompassing administration of anthelmintic drugs will be expected to select resistance alleles within nematode populations. Both human and animal parasitic helminths show a high level of over-dispersion in distribution amongst their hosts (Anderson and Medley, 1985), yet, historically, this has not been taken into account in the design of helminth control programmes. In the veterinary field, targeted control programmes are being promoted to slow the inexorable spread of anthelmintic resistance (Besier, 2008; Stratford et al., 2014a). Targeted anthelmintic drug delivery needs to balance the requirement to control high burdens of pathogenic, life-threatening stages of worms, with the recognition that when treating animals to reduce parasite excretion into the environment, only a relatively small proportion of the population requires treatment. This is particularly the case in managed horse populations in which over-dispersion in faecal egg count (FEC) distribution is high (Lester et al., 2013b; Relf et al., 2013). Such anthelmintic treatment strategies must be underpinned by

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robust evidence regarding the prevalence of the target pathogens present, as well as a detailed knowledge of the status of anthelmintic efficacy across populations. Here, we present a comprehensive study of efficacy of all four broad spectrum anthelmintic drugs across a cohort of UK Thoroughbred stud farms where management practices presumed to be high risk factors for anthelmintic resistance have been performed for decades (Relf et al., 2012). This study was designed to address an important gap in current knowledge regarding anthelmintic sensitivity profiles in breeding Thoroughbred populations by determining the efficacy of ivermectin (IVM), moxidectin (MOX), pyrantel (PYR) and fenbendazole (FBZ) across a number of different sites. This was achieved using the FEC reduction test (FECRT, Coles et al., 1992), supplemented by comparative analysis of different methods for calculating efficacy, and by measurement of strongyle egg reappearance period (ERP) after anthelmintic drug administration. The ERP analysis was undertaken because a shortening in this period after treatment is believed to provide an early indicator of a shift in nematode population sensitivity towards anthelmintic resistance (Sangster, 2001). The knowledge derived from this study will facilitate the development and delivery of control programmes that are based on sound evidence and which aim to help preserve efficacy of any currently effective anthelmintic drugs.

2. Materials and methods

2.1. Study design

Participating Thoroughbred stud farms ($n = 16$) were selected from those enlisted following questionnaire and nematode FEC screening studies (Relf et al., 2012, 2013). These farms were selected on the basis of our previous experience of a high level of compliance in the collection and posting of faecal samples. Each farm also had to contain a minimum of 20 permanent equine residents. All farms were located in England, UK; two in the north, four in the midlands and eight in the south (five of which were in the county of Suffolk). Before being included in the FECRT analyses, samples from horses at each farm were tested by FEC analysis for the presence of nematode (strongyle) eggs. FECs were determined using a modification of a salt-flotation method with a detection limit of one nematode egg per gram of faeces (EPG, Christie and Jackson, 1982). Horses measured as excreting $\geqslant 40$ strongyle EPG at screening were included in the FECRT analysis. At Day 0, immediately before anthelmintic drug administration, freshly voided faecal samples were collected from identified individuals and placed into sealable plastic bags, with all air expelled to prevent egg development. Next, IVM, MOX, PYR or FBZ were administered by the horse owners or managers, at the recommended dose rates of 0.2, 0.4, 19.0 and 7.5 mg/kg of body weight, respectively, with the dose for each individual calculated from an accurate body weight assessed by girth tape or weight scales. IVM and MOX were administered singularly or in combination with praziquantel (PRAZ). The following formulations were administered: 0.2 mg/kg IVM (Eqvalan, Merial Animal Health UK Ltd., UK; Bimectin, Bimeda Animal Health, UK; Animec, Chanelle Animal Health, UK); 0.2 mg/kg IVM and 2.5 mg/kg PRAZ (Eqvalan Duo, Merial Animal Health Ltd., UK); 0.4 mg/kg MOX (Equest, Zoetis UK, Ltd., UK); 0.4 mg/kg MOX and 2.5 mg/kg PRAZ (Equest Pramox, Zoetis UK, Ltd., UK); 19 mg/kg PYR embonate (Strongid P, Elanco Animal Health, UK); 7.5 mg/kg FBZ (Panacur 10% Oral Paste, MSD Animal Health, UK). Horses were categorised according to age (<1 year, $n = 14$; 1 year, $n = 216$; 2–4 years, $n = 63$; $\geqslant 5$ years, $n = 99$). The number of horses tested in each group per farm ranged from six to 30 animals. All faecal samples were posted immediately and once received at the laboratory, stored at approximately 4 °C and analysed within 4 days of collection. Each sample was analysed in duplicate and the mean EPG value per sample determined. Faecal samples were collected and processed in the same manner 14–17 days after anthelmintic drug administration. Eggs were identified as strongyle or *Parascaris equorum* following published guidelines (Thienpont et al., 1986). Laboratory cultures of faecal samples were performed for each individual horse from all participating stud farms and indicated the absence of *Strongylus* spp. at all farms. All FECRTs were undertaken a minimum of 6 weeks after the last administration of IVM, PYR or FBZ and a minimum of 12 weeks after the last administration of MOX.

2.2. Data analysis

FECR was initially calculated for each group of horses (Method 1) using the following formula adapted from the guidelines recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP; Coles et al., 1992), where arithmetic group mean FECs for Day 0 and Days 14–17 were used to estimate group FECRs:

Method 1: Group FECR (%) = ((Day 0 FEC – Day 14 FEC)/Day 0 FEC) × 100

There are no clearly defined, formal guidelines regarding appropriate cut-off limits for determining efficacy within anthelmintic classes in horses based on FECRs (Vidyashankar et al., 2012). The methodology here follows recently published recommendations; i.e. thresholds chosen for establishing appropriate efficacy were arithmetic mean FECRs of >95% for macrocyclic lactone (ML) anthelmintic drugs and >90% for FBZ/PYR anthelmintic drugs (Kaplan and Nielsen, 2010). In addition, 95% lower confidence limits (LCL) were included to give an indication of the range of the data (Vidyashankar et al., 2007; Lester et al., 2013b); non-parametric bootstrapping was used to sample with replacement from the observed pre- and post-treatment FEC, and the lower 2.5 percentiles of 10,000 simulations of group mean FECRs were taken as the LCL (Efron, 1979; Hilborn and Mangel, 1997; Lester et al., 2013b). PopTools software (CSIRO, Australia) was used for bootstrapping (http://www.poptools.org). LCLs of 90% and 80% were selected for classifying resistance to ML and to FBZ/PYR anthelmintic drugs, respectively. Accordingly, if both the percentage of mean FECRs and LCLs fell below the designated cut-offs, anthelmintic resistance was indicated. Alternatively, if either the percentage of mean FECRs or the LCLs fell below these cut-offs, resistance was suggested. The cut-offs used in the current study were selected to reflect original efficacy levels reported in anthelmintic-sensitive strongyle populations of the various active ingredients when they were first registered as veterinary medicines (Cornwell and Jones, 1969; Colglazier et al., 1977; Xiao et al., 1994). Two further methods for estimating the percentage of FECRs were applied to the datasets to inform on their suitability for calculating efficacy. Method 2 involved estimating the group mean percentage of FECRs from arcsine transformed individual proportional reductions (Pook et al., 2002). The proportional reduction in FECs was estimated for each individual ((Day 0 FEC–Day 14 FEC)/Day 0 FEC)) and arcsine transformed. The group mean was then estimated and back-transformed to give group % FECR:

$$\%\text{FECR} = 100 \times (\sin(\text{transformed group mean}))^2$$

The 95% CLs were estimated from the S. E. M. In Method 3, we applied non-parametric bootstrapping (Vidyashankar et al., 2007), where individual proportional reductions were sampled randomly with replacement to estimate the group % FECR and upper and lower 2.5 percentiles of 10,000 simulations were taken as the 95% CLs (Efron, 1979; Hilborn and Mangel, 1997; Lester et al., 2013b).

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