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Short Communication

Searching for ivermectin resistance in Dutch horses

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ABSTRACT

A study was conducted to evaluate the occurrence of resistance against, in particular, ivermectin in cyathostomins in the Netherlands. Seventy horse farms were visited between October 2007 and November 2009. In initial screening, faecal samples were collected 2 weeks after deworming with either ivermectin, moxidectin or doramectin. Pooled faecal samples from a maximum of 10 horses were examined for worm eggs using a modified McMaster technique and for worm larvae after faecal larval cultures. In total 931 horses were involved. On 15 of 70 farms eggs and/or larvae were found. On 8 of these 15 farms a FECRT with ivermectin was performed on 43 horses. Efficacy of ivermectin against cyathostomins of 93% was found in one animal on one farm. Additionally, the strategies and efforts of the horse owners to control cyathostomins, as well as risk factors for the development of macrocyclic lactone resistance were evaluated with a questionnaire. Strikingly, many responders indicated that the control of cyathostomins in horses is achieved through very frequent deworming. Fourteen percent of these owners deworm seven times per year or more. On 34% of the 70 farms treatment was repeated within the Egg Reappearance Period of a product.

1. Introduction

Cyathostomins are the most prevalent horse helminths, with 50 species found in equids worldwide (Lichtenfels et al., 2008). Disease may occur in horses of all ages but young horses (1–4 year old) are more susceptible. To control infections, management measures should be applied (e.g. pasture rotation and reducing grazing density) supported by the use of anthelmintics. Several classes of anthelmintics have been approved for use against cyathostomins in horses. Benzimidazoles were introduced as anthelmintics for horses in the 1960s and pyrantel in 1970. The first representative of the macrocyclic lactones (MLs), ivermectin, was introduced in the early 1980s and moxidectin in the middle of the 1990s. (Pro)benzimidazole resistant cyathostomins are described since the 1960s (Drudge and Elam, 1961) and in the 90s the first reports on pyrantel resistance appeared (Chapman et al., 1996). In 1994 Xiao showed that the efficacy of ML against adult cyathostomins was still 99% (Xiao et al., 1994). Worldwide, several recent studies showed shortening of the Egg Reappearance Period (ERP) (Von Samson-Himmelstjerna et al., 2007; Lyons et al., 2008; Molento et al., 2008) which is considered to indicate resistance (Lyons et al., 2009). Once resistance is present, spreading of the resistant cyathostomin populations is likely by transport of horses.

In the Netherlands, as anywhere else, owners and veterinary practitioners rely heavily on frequent anthelmintic treatments to avoid disease. Therefore, it is not unlikely that reduced ML efficacy also may have developed in the Netherlands. In the present study we conducted a survey to evaluate the presence of ivermectin resistant cyathostomins in Dutch horses. On the same farms, owners were questioned about their worm control practice to determine a possible association between worm control intensity and presence of ML resistance.

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Table 1
Faecal Egg Count Reduction Test and larval culture counts on 43 horses from eight farms. After deworming EPG or LPG was mainly zero for most horses on a farm. Otherwise this was called individual EPG or LPG.

Farm	Number of horses on the farm	Number of FECRT horses	Before deworming				After deworming			
			Mean EPG	EPG range	Mean LPG	LPG range	Mean EPG	Mean LPG	Individual EPG	Individual LPG
1	8	5	130	25–750	108	5–450	0	0	0	1
2	40	3	300	25–700	242	10–450	0	0	–	–
3	24	7	300	50–1350	111	18–1300	3.6	0.014	25	0.1
4	55	12	250	50–1025	456	0–1640	0	0	–	–
5	110	12	444	75–1850	1094	100–3750	0	0	–	–
6	25	1	200	–	30	–	0	0	–	–
7	10	2	1450	700–2200	554	3–1105	0	2.5	0	5
8	70	1	4300	–	520	–	0	0	–	–

2. Materials and methods

2.1. Selection of farms

Between October 2007 and November 2009, 159 horse farms throughout the Netherlands were selected using an internet search. Horse farmers were asked to participate in a trial to evaluate the efficacy of MLs against strongylids. A farm was included if they had three horses or more that were kept on pasture together and the last used wormer was a macrocyclic lactone. Participating farms were included for a visit if deworming fell within one of three periods (October–December period 2007 and 2009 and April–July 2008). Of the 159 approached farms 66 were visited. In addition, 30 veterinary clinics were approached to more efficiently seek farms with positive faecal egg counts after deworming. This led to four more farms that were visited. This study was not designed as a cross-sectional study as farms were not randomly selected.

2.2. Questionnaire

During the first visit, the owners were asked questions about the farm, pasture management and housing, deworming and parasitic problems. This was based on the questionnaire of Osterman Lind et al. (2007).

2.3. Screening

Fourteen days after deworming 70 farms were visited for the first time to collect faecal samples. The samples were pooled with a maximum of 10 horses per sample (Eysker et al., 2007). On several larger farms faecal material for more than one pooled sample (2–6) was collected. In total 115 pooled faecal samples were examined. The decision on how the samples were pooled depended on how the groups of horses were maintained on pasture.

Pools consisted of 3.0 g from each individual sample and these were mixed thoroughly. A modified McMaster method with saturated NaCl solution (MAFF, 1986) was done with 3.0 g faeces from the pooled sample and 4 chambers were counted per pooled sample, resulting in a detection limit of 25 eggs per gram of faeces (EPG). For the pooled cultures each horse contributed equal amounts of faeces to a thoroughly mixed sample from which 25 g was cultured in duplicate in glass jars for 10–12 days at 21 °C (Borgsteede and Hendriks, 1973). After the culture period the jars were filled with tap water and turned upside down on Petri dishes. The following day the larvae were collected, differentiated (Thienpont et al., 2003) and counted to determine the numbers of larvae per gram faeces (LPG), with an overall detection limit of 0.04 LPG (or 0.4 LPG per individual horse).

2.4. Faecal Egg Count Reduction Test (FECRT)

If screening showed a positive EPG and/or LPG the second and third visit to the farm were planned in order to perform a FECRT. The second visit was planned after the assumed ERP period had elapsed. Faeces from individual horses was examined with the McMaster technique, as well as that 25 g of faeces from each horse was cultured as described above. Examinations were done on day 0 and day 14 after ivermectin treatment (Eqvalan® or Equimectin®, 200 μg per kg bodyweight. Bodyweight (BW) was estimated using a girth tape (Coles et al., 2006). In some cases, the weight was estimated visually by two people followed by adding 10% to the mean of both visual estimations. This was done by a trained student and the owner because we felt the girth measurement in certain breeds underestimates the actual weight. An efficacy of less than 95% for an individual animal indicated suspected resistance (Craven et al., 1998; Coles et al., 2006). We were able to perform a FECRT on 8 from the in total 15 farms with positive egg counts or larval cultures that were found within the screening.

3. Results

3.1. Screening and FECRT

Fifteen farms (16 pools) had a positive EPG and/or LPG 14 days after deworming. On 8 of these farms an FECRT was performed on individual animals with positive EPG's. Taken together, 931 horses were investigated within the screening procedure and an FECRT was performed on 43 horses. EPG and LPG reduction was 100% on 6 farms (Table 1). On farm 3 (Table 1) a yearling with a reduction in EPG of 93%