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Occurrence of fenbendazole resistance in *Parascaris* spp. on breeding farms in Sweden

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ABSTRACT

Anthelmintic resistance is an increasing problem in many gastrointestinal parasites of grazing animals. Among these, the equine roundworm, *Parascaris* spp., has developed wide-spread resistance to macrocyclic lactones over the past decades. Additionally, there are recent observations of emerging treatment failure of both tetrahydropyrimidine and fenbendazole. Therefore, the aims of this study were to further investigate the occurrence of fenbendazole resistance on breeding farms and to explore potential management-related risk factors associated with resistance in *Parascaris* spp. in Sweden. Eleven farms with 92 foals positive for *Parascaris* spp. were included in a faecal egg count reduction test during the years 2021–2023. According to the clinical protocol of the guidelines of the World Association for the Advancement of Veterinary Parasitology, fenbendazole resistance was present on four farms with efficacies varying from 45 % to 96 %. Having previously reported reduced efficacy on one of these farms, we can now confirm that fenbendazole resistance in *Parascaris* spp. has established. Farms with more than 40 yearly born foals had a significantly higher probability of having resistant *Parascaris* spp. Populations compared with smaller farms, (generalized linear model (GLM), $t = 70.39$, $p < 0.001$). In addition, there was a correlation between the number of foals on the farm and the frequency of yearly treatments showing that farms with < 20 foals were notably inclined to administer treatments twice during the first year (GLM, $t=2.76$, $p < 0.05$) in contrast to larger farms with > 40 foals that were using more frequent treatment intervals. In conclusion, this study confirms the establishment of fenbendazole resistance in *Parascaris* spp. populations on Swedish stud farms with the number of foals on the farm identified as a risk factor for development of anthelmintic resistance.

1. Introduction

The equine roundworms, *Parascaris equroum* and *Parascaris univalens* are common parasites of foals around the world and have traditionally been referred to as *P. equorum.* However, recent studies have suggested that *P. univalens* is the dominating species infecting horses (Jabbar et al., 2014; Nielsen et al., 2014; von Samson-Himmelstjerna et al., 2021, Martin et al., 2021). In most foals *Parascaris* infection is subclinical or causes mild symptoms such as weight loss, impaired growth, coughing and nasal discharge (Clayton and Duncan, 1978). However, if a foal is heavily infected, the mass of worms in the small intestine can cause severe colic and even rupture of the intestinal wall (Cribb et al., 2006). Due to the potential pathogenicity of the parasite, most treatment guidelines instruct to treat foals at regular intervals, two to four times during the foal`s first year of life (ESCCAP, 2019; Nielsen et al., 2019; Rendle et al., 2019). Ideally, treatment should be monitored through regular faecal diagnostics at 2, 5, and 8 months of age (ESCCAP, 2019). In Sweden, the current recommendation is routine treatment at 2–2.5 months and 4–4.5 months of age, followed by faecal diagnostics after weaning and an additional treatment of positive foals (Hedberg Alm et al., 2022).

Historically, several anthelmintic drug classes have been viable options for treatment of *Parascaris* spp. in horses, but since the beginning of the 21st century, there have been a number of reports of resistance to macrocyclic lactones (Boersema et al., 2002; Peregrine et al., 2014). This development has led to a change in the compound of choice for the treatment of *Parascaris* spp. to fenbendazole and pyrantel. However, treatment failure of both these drug classes has also been reported in recent years (Armstrong et al., 2014; Alanazi et al., 2017; Martin et al., 2018; Martin et al., 2021). This development of multi-resistance is an increasing threat to foal health and the equine industry as *Parascaris* spp. is a potentially lethal parasite.

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A study performed on eleven Swedish stud farms in 2010 showed that fenbendazole had efficacies of 100 % against *Parascaris* spp. (Tydén et al., 2014). In 2017, a similar study was performed to evaluate the efficacy of fenbendazole and to karyotype *Parascaris* spp. in Sweden. This study identified only *P. univalens* and, indicated reduced efficacy on two farms, due to treatment failure in three foals (Martin et al., 2018). The fenbendazole efficacy was reinvestigated on one of the farms with indicated emergence of resistance in 2019 and 2020 and reached efficacies of 78 % and 73 %, respectively. In addition, 38 % of the foals excreted *P. univalens* eggs post fenbendazole treatment in 2019, and 74 % of the foals in 2020 (Martin et al., 2021). Together these studies imply that anthelmintic resistance to fenbendazole has started to emerge in Swedish *P. univalens* populations.

Risk factors for infection with *Parascaris* spp. were investigated by Hautala et al. (2019) and showed that young horses on large breeding farms had a higher probability of acquiring a patent infection. In addition, regular movements of horses between farms further increased the probability of patent *Parascaris* spp. infection in the foals. Another study showed that keeping the foals on deep litter bedding increased the risk of *Parascaris* spp. infection (Fritzen et al., 2010). However, risk factors involved in development of anthelmintic resistance in *Parascaris* spp. have so far been poorly investigated.

The aims of this study were to further investigate the occurrence of fenbendazole resistance on breeding farms and to explore potential management-related risk factors associated with the occurrence of resistance in *Parascaris* spp. in Sweden.

2. Materials and methods

2.1. Study design

Since karyotyping was not performed in the present study, *Parascaris* spp. is used throughout. The study was conducted during three years (2021–2023). Stud farms were contacted and asked to participate in the project and farms with at least four foals younger than one year and excreting $\geq$ 50 *Parascaris* spp. eggs per gram faeces (EPG) were considered eligible for inclusion. Faecal samples were analysed pre-treatment and 9–16 days post-treatment with fenbendazole (Axilur® vet. 19 %, MSD Animal Health, Sweden) oral paste 7.5 mg/kg body weight. The weights of the foals were estimated using a weight tape (Boehringer Ingelheim AB, Sweden) and rounded up to the nearest 50 kg to avoid giving an insufficient dose of the drug. The foals in this study were born between January and May. Depending on their age and season/temperature of the year, the mares and their foals were stabled indoors in a separate box on either straw or shavings, which were cleaned out every morning. At the time of the study the foals were out on field 24 h a day. Unfortunately, we do not have any information about the disinfection routines applied on the farms. Written owner consent was obtained for all included foals.

2.2. Faecal analysis

Paired faecal egg counts (FECs) were performed prior and post-treatment using a modified McMaster technique (Coles et al., 1992). In brief, 6 g of each faecal sample were floatated using saturated NaCl solution (SG = 1.18 g/cm^2) and for each sample two McMaster slides were analysed, resulting in a multiplication factor of 12.5.

Two exceptions of the analysis of faecal samples occurred. Samples from farm 1 were performed by the parasitology laboratory at the Swedish Veterinary Agency (SVA, Sweden) and 18 of the 24 pre-treatment samples from farm 7 were analysed at Vidilab AB (Sweden). Both SVA and Vidilab AB are accredited parasitology laboratories and use a modified McMaster method with a multiplication factor of 50.

2.3. Data analysis

The results were analysed in two ways:

i) To classify the egg count reductions as resistant or susceptible according to the current guidelines provided by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Kaplan et al., 2023) and the online tool at www.fecrt.com (Denwood et al., 2019, 2023) according to the clinical protocol due to small number of individuals per farm and low egg counts. A control group, left untreated, was not possible to include, as the foals were privately owned and adhered to keep to the regular deworming routines on the farm. Thus, resistance was conferred if the upper 90 % credible interval was less than the 99.9 % target efficacy of fenbendazole in *Parascaris* spp.. Since all foals on susceptible farms had a post-treatment faecal egg count of zero, precluding the use of delta method, susceptibility to fenbendazole was conferred if a significant p-value was obtained by the Beta Negative Binomial (BNB) method (Denwood et al., 2019, 2023).
ii) For descriptive purposes, the egg reduction, or efficacy, was calculated using a Bayesian hierarchical model on the shiny-eggCounts web interface, version 2.3–2 (Torgerson et al., 2014).

2.4. Questionnaire data

All participating farms were interviewed by the first author regarding number of residing foals and horses, *Parascaris* spp. treatment routines, pasture management and routines of new arrival of foals on the premises according to a standardized questionnaire (Supplementary file 1). The faecal egg count reduction test (FERCT) result from each farm, resistant or susceptible, was used as respondent variable in a generalized linear model (GLM) to investigate possible associations between farm resistance status and the questionnaire responses "total number of foals on the farm", "anthelmintic routine" and "number of treatments in the first year". In addition, a possible association between the number of foals on the farm and the number of anthelmintic treatments given was investigated using a GLM. To account for overdispersion, a quasibinomial error distribution was used. The statistical analysis was conducted in R v4.3.2 (R Core Team, 2023).

3. Results

After initial screening of faecal samples from 21 farms, eleven stud farms with a total of 92 foals less than 12 months old were included in the FECRT. The number of foals included on each farm, their age, the year the study was performed and treatments during the first year of life for each foal on the farm are shown in Table 1.

3.1. Faecal egg count reduction test

The mean and range FECs pre- and post-treatment, total number of eggs counted, proportion of horses shedding eggs post-treatment, efficacy and classification are shown in Table 1 and for individual values see supplementary file 1. Four farms (farm 1, 7, 10 and 11) were classified as resistant using the clinical protocol in the WAAVP-guidelines (Kaplan et al., 2023), with the 90 % upper credible limit being less than the expected efficacy of 99.9 % as calculated by the delta method (Denwood et al., 2019, 2023). These farms also showed reduced efficacies of 83 %, 45 %, 84 % and 96 % respectively according to the Bayesian hierarchical model (Torgerson et al., 2014). Several foals on these farms were shedding eggs post-treatment; 67 % of the foals on farm 1, 83 % on farm 7, 62 % on farm 10 and 50 % on farm 11.

The remaining seven farms were classified as susceptible ($p < 0.001$) with efficacies of 100 % according to the Bayesian hierarchical model (Torgerson et al., 2014). As all horses on these farms were negative for *Parascaris* spp. eggs in the post-treatment samples, a BNB distribution

Table 1
Included stud farms, sorted by the year FECRT was performed, number of participating foals, total number of foals on the farm, age of participating foals, number of FBZ treatments the first year, mean (range) EPG pre- and post-treatment, total eggs counted, proportion of horses excreting eggs post treatment, efficacy according to Bayesian hierarchical model (Torgerson et al., 2014) and classified as either susceptible (S) or resistant (R) (90–99.9) according to the clinical protocol of the WAAVP guidelines (Kaplan et al., 2023).

Farm no	Year tested	Participating foals (total foals at the farm)	Age of included foals (months)	Number of FBZ treatments first year	Mean (range) EPG pre treatment	Mean (range) EPG post treatment	Total no of eggs counted	% horses excreting eggs post treatment	Efficacy % (UCL-LCL)[d]	Classification incl UCL and LCL for R[e] and p for S[f] at two weeks post-treatment
1	2021	9 (40)	< 12[a]	6	611 (350–1350)	106 (0–350)	110[b]	67 %	83 % (81.5–83.9)	R 66.3 % - 94.2 %
2	2021	6 (10)	6–8	2–3	457 (50–1513)	0 (0–0)	219	-	100 % (99.9–100)	S p< 0.001
3	2021	6 (20)	7–8	2–3	267 (125–588)	0 (0–0)	128	-	100 % (99.9–100)	S p< 0.001
4	2021	5 (9)	6–9	2	553 (63–1250)	0 (0–0)	221	-	100 % (99.9–100)	S p< 0.001
5	2022	4 (10)	6–9	2	204 (63–388)	0 (0–0)	65	-	100 % (99.9–100)	S p< 0.001
6	2022	6 (16)	4–5	2	271 (75–975)	0 (0–0)	130	-	100 % (99.9–100)	S p< 0.001
7	2022	23 (60)	4–7	2–3	1164 (100–6150)	642 (0–4038)	536[c]	83 %	45 % (43.7–45.9)	R −3.9 % - 79.7 %
8	2023	5 (10)	7–9	2	435 (125–663)	0 (0–0)	174	-	100 % (99.8–100)	S p< 0.001
9	2023	5 (20)	7–10	2	295 (138–550)	0 (0–0)	118	-	100 % (99.8–100)	S p < 0.001
10	2023	13 (50)	5–8	4–5	320 (138–813)	51 (0–225)	333	62 %	84 % (82.5–85.3)	R 72.5 % - 92.8 %
11	2023	10 (80)	6–8	2–3	1993 (275–4900)	75 (0–538)	1594	50 %	96 % (95.9–96.5)	R 89.9 % - 99.6 %

[a] Exact age of the foals were not stated
[b] Analysed at Swedish Veterinary Agency, multiplication factor 50
[c] 18 of the pre-treatment samples analysed at Vidilab AB, multiplication factor 50
[d] Calculated by the Bayesian hierarchical model using https://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/ (Torgerson et al., 2014)
[e] Calculated by the delta method using https://fecrt.com (Denwood et al., 2019, 2023)
[f] Calculated by the BNB method using https://fecrt.com as there were no eggs detected post-treatment (Denwood et al., 2019, 2023)

was used for the statistical calculation (Denwood et al., 2019, 2023).

3.2. Questionnaire results

All questionnaire responses, including free text, can be found in supplementary file 3. Total number of horses and foals on each farm and fenbendazole treatments during the first year of foals can be found in Table 1. Answers regarding anthelmintic routines and pasture management are summarised in Table 2.

3.2.1. Number of foals on the farm

There was a significant association between the total number of foals on the farm and the resistance classification, as farms with > 40 foals had a significantly higher probability of having resistant *Parascaris* spp. populations (GLM, $t = 70.39$, $p < 0.001$).

3.2.2. Anthelmintic routines

The anthelmintic routine used for treatment of *Parascaris* spp. varied between the farms (Table 2). Fenbendazole was used as the sole drug on all farms but two that instead rotated between fenbendazole and pyrantel. The average number of anthelmintic treatments on the farms varied between two and six treatments during the foals ́first year of life (Table 2). There was an association between the number of resident foals on the farm and the estimated number of treatments, where farms conducting more than two anthelmintic treatments per year were significantly larger in terms of number of resident foals compared to farms conducting no more than two treatments during the foals` first year (GLM, $t=2.76$, $p < 0.05$) (Fig. 1).

Four farms evaluated the efficacy of *Parascaris* spp. treatment, three farms on yearly basis and one farm at more infrequent intervals. The remaining farms did not test the treatment efficacy.

Personnel at two of the resistant farms (no 1 and 10) had observed signs of reduced efficacy of fenbendazole due to foals acquiring *Parascaris* spp. induced impaction despite being treated according to plan (Table 1, farm 1 and 10). Furthermore, reduced efficacy was detected through regular FECRT at the third resistant farm (Table 1, farm 7). Farm 11, did not observe any signs of reduced efficacy.

3.2.3. Pasture management and new arrivals

The majority of farms (10 of 11 farms) used the same pastures for the foals every year and none of the farms regularly removed faeces from the pastures. Management routines applied were co-grazing or bi-annual rotational grazing with ruminants (2 of 11 farms) or ploughing and reseeding the pastures every 3–7 years (6 of 11 farms) to lower the infection pressure of parasites (Table 2).

Different routines were applied for new mares and foals arriving at the farm. Five farms never mixed new arrivals with the existing herd, three farms treated new arrivals with an anthelmintic against *Parascaris* spp., two farms kept new arrivals in a separate field for two to three weeks before turn out with the residing horses, although without any parasite diagnostics or anthelmintic treatments, and one farm did not apply (Table 2).

3.2.4. Owner attitudes

Six of the ten responding farms expressed concern about anthelmintic resistance. Some of these farms aimed to reduce the frequency of treatments for foals and horses and to improve pasture management practises due to the emerging parasitic resistance. They also highlighted the impact of education regarding the timing of faecal testing of horses for parasites, which faecal analysis to perform and the appropriate anthelmintic drug choice as well as the dissemination of treatment recommendations and research results to both horse owners, stud farms and veterinarians.

Table 2
Questionnaire data collected from participating farms. For Q2 - total number of foals, see Table 1. All answers can be found in supplementary file 1.

Question	Response alternative	Response
Q3. Anthelmintic routine for *Parascaris* sp.	i) routinely at week 8–10 and 16–18 ii) routinely at week 8–10, 16–18 and after weaning if detected at faecal diagnostics iii) routinely at week 8–10 and 16–18 and after weaning iv) treatment only if detected at faecal diagnostics v) own treatment plan described in free text.	3 farms 1 farm 2 farms 1 farm 4 farms
Q4. Estimated number of treatments with FBZ during the foals first year.	i) 2 ii) 2–3 iii) 4–5 iv) 6	5 farms 4 farms 1 farm 1 farm
Q5[a]. Anthelmintic substances used to treat *Parascaris* sp.	i) FBZ ii) PYR + FBZ	9 farms 2 farms
Q6. Have seen signs of reduced efficacy of FBZ.	i) Yes ii) No	3 farms 8 farms
Q7. Regular control of FBZ efficacy. If yes, how often?	i) Yes, free text ii) No	4 farms 7 farms
Q8. Use of same pastures for foals every year.	i) Yes ii) No	10 farms 1 farm
Q9 Faecal removal from pastures.	i) Yes ii) No	0 farm 11 farms
Q10[a,b] Other pasture management routines.	i) harrowing ii) bi-annual rotation or co-grazing with ruminants iii) ploughing/reseeding, time frame in free text iv) other - free text	0 farm 3 farms 6 farms 1 farm
Q11 Management of new arrivals (foals).	i) kept in separate fields ii) faecal sampling iii) treatment with anthelmintic drug iv) drug treatment and control of efficacy v) no special routines vi) other	5 farms 0 farm 3 farms 0 farm 1 farm 2 farms
Q12[b] Are you worried about anthelmintic resistance in parasites? If yes, have this affected your routines?	i) Yes, free text ii) No	6 farms 4 farms
Q13[b] Is more information needed about anthelmintic resistance?	i) Yes ii) No	10 farms 0 farm

[a] The respondents could choose more than one alternative for Q5 and Q10, and the answers should be read as percentage of respondents answering that they did that/those alternative(s).
[b] Q10, Q12, Q13 were answered by 10 of 11 farms.

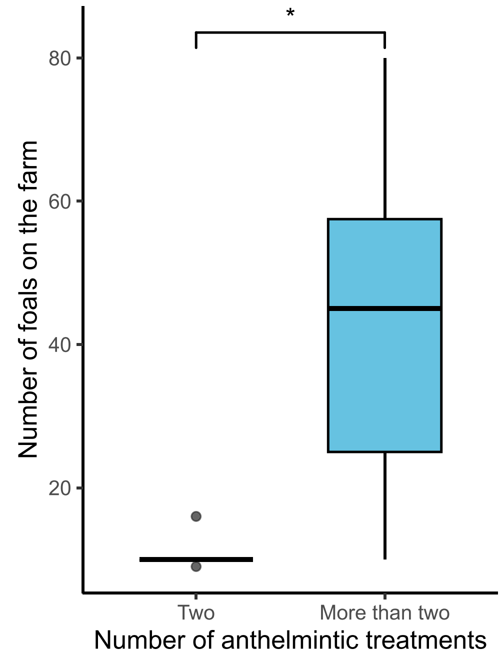

Fig. 1. The number of foals on the farm affects the number of anthelmintic treatments administered to the foals during the first year, where farms (n=5) administering two anthelmintic treatments had fewer foals than those (n=6) administering more than two treatments ($p < 0.05$). Dots in the boxplot indicates outliers.

4. Discussion

The results of this present study show that fenbendazole resistance in *Parascaris* spp. is present on a considerable proportion of breeding farms in Sweden. Four of the eleven included farms were classified as resistant according to the clinical protocol in the currently accepted guidelines from the WAAVP (Kaplan et al., 2023). In addition, the efficacies of fenbendazole treatment on these farms varied between 45 % and 96 % and at least half of the foals on each farm excreted *Parascaris* spp. eggs post treatment. Still, the majority, seven farms, were classified as susceptible and showed 100 % treatment efficacy. Although this data set was small, the presence of a high number of foals on the farm was found to be a significant risk factor ($p < 0.001$) for the emergence of fenbendazole resistance in *Parascaris* spp. Farms showing resistance typically housed more than 40 foals, while susceptible farms tended to have less than 20 foals. A similar association with the development of resistance in Cyathostominae has been observed by Salle et al. (2017), where a low stocking density (<5 horses/ha) was associated with a reduced risk of anthelmintic resistance. In addition, a previous study observed an increased risk of *Parascaris* spp. infection in foals on larger breeding farms, which was defined as at least four foals born each year (Hautala et al., 2019). In comparison, the smallest breeding farm in our study had nine foals born the year they were included in the FECRT.

Previous reports in Australia, the Middle East and Europe have highlighted instances of reduced efficacy of fenbendazole in *Parascaris* spp. (Armstrong et al., 2014; Alanazi et al., 2017; Martin et al., 2021). In addition, there have been reports of reduced efficacy of benzimidazole drugs in other closely related ascarid roundworms such as *Ascaris lumbricoides*, *Ascaridia galli*, and *Ascaridia dissimilis* (Krucken et al., 2017; Collins et al., 2019; Höglund et al., 2023; Gebreyesus et al., 2024). Together with the results of the current study, this suggests that benzimidazole resistance is an increasing problem in ascarids. Further, this implies that there may be ongoing development of fenbendazole resistance in *Parascaris* spp. in other geographical regions, yet remaining undetected due to limited studies.

An interesting observation in the present study is the continuous reduction in efficacy on farm 7, which we have studied during several years, and hence confirmed the presence of a truly resistant *Parascaris* spp. population. The efficacies have dropped from 100 % in 2010 (Tydén et al., 2014) to 88 % in 2017 (Martin et al., 2018), 78 % in 2019, 73 % 2020 (Martin et al., 2021) and 45 % in the current study (2022). This farm does not follow the national guideline for *Parscaris* spp. treatment of foals. Instead, this farm has implemented their own monitoring programme by routinely analysing faecal samples every fourth week. On this farm, any foals excreting ≥ 100 EPG of *Parascaris* spp. are treated with fenbendazole. This strategy resulted in an increasing number of treatments of foals with treatment failure, leading to an increased selection for resistance. Therefore, it can be inferred that once anthelmintic resistance has developed within a *Parascaris* spp. population, efficacy continues to decline over time. This trend is consistent with the pattern observed in the development of anthelmintic resistance in other parasitic nematodes (Sangster, 1999).

The current WAAVP guidelines recommend including a minimum of five foals from each farm (Kaplan et al., 2023). This recommendation was met on all farms but farm 5, where only four foals were positive for *Parascaris* spp. in the pre-treatment sample. It is also suggested in the guidelines to only include foals with pre-treatment FECs of 100 or more due to the risk of coprophagia causing positive egg counts below 100 (Kaplan et al., 2023). However, we included five foals in the FECRTs, despite having FECs of 50–75 pre-treatment, to reach the recommended number of individuals/farm. Coprophagia cannot be ruled out in these individuals with low egg counts pre-treatment and might have resulted in a falsely susceptible analysis on farms 2, 4, 5, and 6. Despite this, all remaining foals on these farms were also negative post-treatment. In addition, farms 10 and 11, classified as resistant, had five out of eight foals and three out of five foals, with egg counts below 100 EPG, respectively. Including these individuals with low egg count might have skewed the resistant classification. Another criterion in the guideline that was not met was the inclusion of an untreated control group. (Kaplan et al., 2023). Because, privately owned horses were included in this study, it was not feasible to incorporate an untreated control group, as horse owners were unwilling to refrain from treating their animals. However, not including a control group of foals with *Parascaris* spp. infection can result in an overestimate of drug efficacy due to evolving immunity to the parasite with age (Clayton and Duncan, 1978, Craig et al., 2007).

An updated national guideline for the treatment of *Parascaris* spp. has recently been published and is freely available in Sweden, recommending to treat foals routinely at 8–10 weeks and 16–18 weeks of age and thereafter depending on results from faecal samples after weaning (Hedberg Alm et al., 2022). However, these guidelines were only followed by one of the participating farms. Of the remaining farms, three farms treated at 8–10 weeks and 16–18 weeks of age, and the rest regularly performed treatments between three to six times during the foals' first year. Smaller farms (median >10 foals yearly) were more inclined to administer two treatments within the first year, in contrast to larger farms (median 45 foals yearly) that were more likely to treat more than two times. However, due to large variations in treatment routines between farms, there was no statistically significant association between treatment routines and fenbendazole resistance. Even though selective treatment has been shown to be associated with higher drug efficacy in Cyathostominae (Salle et al., 2017). *Parascaris* spp. is one of the few parasites where routine treatment is recommended as most foals are presumed to be infected (ESCCAP, 2019; Nielsen et al., 2019; Rendle et al., 2019). A computational model by Leathwick et al. (2017) suggests that the number of treatments and the timing of the treatments affect the development of resistance in *Parascaris* spp., with fewer treatments during the first six months of the foals life leading to decreased risk of resistance. Since an overuse of anthelmintic drugs selects for resistant parasites (Sangster, 1999; Falzon et al., 2014) it could be speculated that farms that have designed their own treatment routines with more frequent treatments of *Parascaris* spp., could have a higher likelihood of resistance development.

In conclusion, this study confirms the establishment of fenbendazole resistance in *Parascaris* spp. populations on Swedish stud farms. Given the widespread development of resistance to macrocyclic lactones and the demonstrated resistance to pyrantel in the Swedish *Parascaris* spp. population (Martin et al., 2018), fenbendazole is currently the drug of choice. The present results are therefore alarming, as *Parascaris* spp. has the potential to cause severe harm with possible lethal outcomes, underscoring the critical need for effective drugs to control infection. Even though several of the participating farms expressed concern about anthelmintic resistance, only four farms performed regular efficacy testing post-treatment of *Parascaris* spp. With the emergence of multi-resistant *Parascaris* spp. populations, critical investigations of novel treatment and control methods, such as enhanced pasture management, needs to be urgently explored for this parasite.

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CRediT authorship contribution statement

Eva Tydén: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Frida Martin:** Writing – original draft, Methodology, Formal analysis, Data curation. **Peter Halvarsson:** Writing – review & editing, Visualization, Formal analysis. **Ylva Hedberg Alm:** Writing – review & editing, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Frida Martin reports financial support, article publishing charges, and statistical analysis were provided by Swedish-Norwegian Foundation for Equine Research. Eva Tyden reports financial support, article publishing charges, and statistical analysis were provided by Swedish-Norwegian Foundation for Equine Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110272.

References

Alanazi, A.D., Mukbel, R.M., Alyousif, M.S., AlShehri, Z.S., Alanazi, I.O., Al-Mohammed, H.I., 2017. A field study on the anthelmintic resistance of *Parascaris* sppsppp. in Arab foals in the Riyadh region, Saudi Arabia. Vet. Q 37, 200–205.
Armstrong, S.K., Woodgate, R.G., Gough, S., Heller, J., Sangster, N.C., Hughes, K.J., 2014. The efficacy of ivermectin, pyrantel and fenbendazole against *Parascaris equorum* infection in foals on farms in Australia. Vet. Parasitol. 205, 575–580.
Boersema, J.H., Eysker, M., Nas, J.W.M., 2002. Apparent resistance of *Parascaris equorum* to macrocyclic lactones. Vet. Rec. 150, 279–281.
Clayton, H.M., Duncan, J.L., 1978. Clinical signs associated with *Parascaris equorum* infection in worm-free pony foals and yearlings. Vet. Parasitol. 4, 69–78.
Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.
Collins, J.B., Jordan, B., Baldwin, L., Hebron, C., Paras, K., Vidyashankar, A.N., Kaplan, R.M., 2019. Resistance to fenbendazole in *Ascaridia dissimilis*, an important nematode parasite of turkeys. Poult. Sci. 98, 5412–5415.
Craig, T.M., Diamond, P.L., Ferwerda, N.S., Thompson, J.A., 2007. Evidence of ivermectin resistance by Parascaris equorum on a Texas horse farm. J. Equine Vet. Sci. 27, 67–71.
Cribb, N.C., Cote, N.M., Boure, L.P., Peregrine, A.S., 2006. Acute small intestinal obstruction associated with *Parascaris equorum* infection in young horses: 25 cases (1985-2004). N. Z. Vet. J. 54, 338–343.
Denwood, M., 2023. 〈www.fecrt.com〉, 2023-08-21.
Denwood, M.J., Innocent, G.T., Prentice, J.C., Matthews, L., Reid, S.W.J., Pipper, C.B., Levecke, B., Kaplan, R.M., Kotze, A.C., Keiser, J., Palmeirim, M., McKendrick, I.J., 2019. A hypothesis testing framework for the ratio of means of two negative binomial distributions: classifying the efficacy of anthelmintic treatment against intestinal parasites. arXiv pre Print. Serv. 〈https://doi.org/10.48550/arXiv.1910.06667〉.
Denwood, M.J., Kaplan, R.M., McKendrick, I.J., Thamsborg, S.M., Nielsen, M.K., Levecke, B., 2023. A statistical framework for calculating prosppective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine. Vet. Parasitol. 314, 109867.

ESCCAP, 2019. A guide to the treatment and control of equine gastrointestinal parasite infections. European Scientific Councel Companion Animal Parasites, ESCCAP Guideline 08 Second Edition – March 2019.

Falzon, L.C., O'Neill, T.J., Menzies, P.I., Peregrine, A.S., Jones-Bitton, A., vanLeeuwen, J., Mederos, A., 2014. A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. Prev. Vet. Med 117, 388–402.

Fritzen, B., Rohn, K., Schnieder, T., von Samson-Himmelstjerna, G., 2010. Endoparasite control management on horse farms–lessons from worm prevalence and questionnaire data. Equine Vet. J. 42, 79–83.

Gebreyesus, T.D., Makonnen, E., Tadele, T., Mekete, K., Gashaw, H., Gerba, H., Aklillu, E., 2024. Reduced efficacy of single-dose albendazole against *Ascaris lumbricoides*, and *Trichuris trichiura*, and high reinfection rate after cure among school children in southern Ethiopia: a prosppective cohort study. Infect. Dis. Poverty 13, 8.

Hautala, K., Nareaho, A., Kauppinen, O., Nielsen, M.K., Sukura, A., Rajala-Schultz, P.J., 2019. Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantel embonate against *Parascaris* spp. Vet. Parasitol. 273, 52–59.

Hedberg Alm, Y., Tydén E., Riihimäki, M., Anlén, K., Nyman S., Hedenby, J., Osterman Lind, E., Wartel, M., Svedberg, P. 2022. Hästens mag-tarmparasiter - Att förebygga och behandla.

Höglund, J., Daş, G., Tarbiat, B., Geldhof, P., Jansson, D.S., Gauly, M., 2023. Ascaridia galli - An old problem that requires new solutions. Int. J. Parasitol. Drugs Drug Resist. 23, 1–9.

Kaplan, R.M., Denwood, M.J., Nielsen, M.K., Thamsborg, S.M., Torgerson, P.R., Gilleard, J.S., Dobson, R.J., Vercruysse, J., Levecke, B., 2023. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Vet. Parasitol. 318, 109936.

Krucken, J., Fraundorfer, K., Mugisha, J.C., Ramunke, S., Sifft, K.C., Geus, D., Habarugira, F., Ndoli, J., Sendegeya, A., Mukampunga, C., Bayingana, C., Aebischer, T., Demeler, J., Gahutu, J.B., Mockenhaupt, F.P., von Samson-Himmelstjerna, G., 2017. Reduced efficacy of albendazole against *Ascaris lumbricoides* in Rwandan schoolchildren. Int J. Parasitol. Drugs Drug Resist 7, 262–271.

Leathwick, D.M., Sauermann, C.W., Geurden, T., Nielsen, M.K., 2017. Managing anthelmintic resistance in *Parascaris sppsppp.*: a modelling exercise. Vet. Parasitol. 240, 75–81.

Martin, F., Halvarsson, P., Delhomme, N., Höglund, J., Tydén, E., 2021. Exploring the beta-tubulin gene family in a benzimidazole-resistant *Parascaris univalens* population. Int J. Parasitol. Drugs Drug Resist 17, 84–91.

Martin, F., Höglund, J., Bergström, T.F., Karlsson Lindsjö, O., Tydén, E., 2018. Resistance to pyrantel embonate and efficacy of fenbendazole in *Parascaris univalens* on Swedish stud farms. Vet. Parasitol. 264, 69–73.

Nielsen, M.K., Mittel, L., Grice, A., Erskine, M., Graves, E., Vaala, W., Tully, R.C., French, D.D., Bowman, R., Kaplan, R.M., 2019. AAEP Parasite Control Guidelines. American Association of Equine Practitioners, Lexington. ⟨www.aaep.org⟩.

Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic resistance in important parasites of horses: does it really matter? Vet. Parasitol. 201, 1–8.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨https://www.R-project.org/⟩.

Rendle, D., Austin, C., Bowen, M., Cameron, I., Furtado, T., Hodgkinson, J., McGorum, B., Matthews, J., 2019. Equine de-worming: a consensus on current best practice. UK-Vet. Equine 3, 1–14.

Salle, G., Cortet, J., Bois, I., Dubes, C., Guyot-Sionest, Q., Larrieu, C., Landrin, V., Majorel, G., Wittreck, S., Woringer, E., Courouce, A., Guillot, J., Jacquiet, P., Guegnard, F., Blanchard, A., Leblond, A., 2017. Risk factor analysis of equine strongyle resistance to anthelmintics. Int J. Parasitol. Drugs Drug Resist 7, 407–415.

Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. Int J. Parasitol. 29, 115–124.

Torgerson, P.R., Paul, M., Furrer, R., 2014. Evaluating faecal egg count reduction using a sppecifically designed package "eggCounts" in R and a user friendly web interface. Int. J. Parasitol. 44, 299–303.

Tydén, E., Dahlberg, J., Karlberg, O., Höglund, J., 2014. Deep amplicon sequencing of preselected isolates of *Parascaris equorum* in beta-tubulin codons associated with benzimidazole resistance in other nematodes. Parasit. Vectors 7, 410.