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Retained efficacy of ivermectin against cyathostomins in Swedish horse establishments practicing selective anthelmintic treatment

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ABSTRACT

Cyathostominae are ubiquitous to grazing horses and regarded the most prevalent internal parasite in the horse. Unfortunately, decades of indiscriminate use of anthelmintic drugs have resulted in the development of resistance in cyathostomins to all currently available drug groups, the most recent being a documented lack of efficacy to the macrocyclic lactones (ML). *In vivo* determination of anthelmintic resistance in horses most often utilises the faecal egg count reduction test (FECRT). Further, a shortened egg reappearance period (ERP) can indicate a change in response to the applied treatment and suggest an upcoming reduction of efficacy. Although both true resistance as demonstrated by the FECRT and shorter ERPs after ML treatment have now been shown in cyathostomins worldwide, the efficacy of ML as regards to cyathostomins in Sweden is currently unknown. The aim of the present study was therefore to determine FECRTs and ERPs after ivermectin (IVM) treatment in Swedish horses. Sixteen equestrian establishments with a minimum of six horses excreting at least 150 eggs per gram faeces (EPG) at screening were selected. For each establishment, FECRTs and ERPs were determined by collecting faecal samples prior to and 14 days after IVM treatment (200 μg/kg), and thereafter at weekly intervals for a total of eight weeks. All participants responded to a questionnaire detailing pasture management methods and anthelmintic routines.Questionnaire results showed that the majority of establishments (69%) only treated horses with anthelmintic drugs if indicated by faecal diagnostics and all of the establishments had a mean FECRT exceeding 99.0% and ERPs ranging from six to over eight weeks. The ERP was shown to increase with age as young individuals were shown to excrete cyathostomin eggs earlier after treatment compared with older horses ($R = 0.21$, $p = 0.015$). Riding schools, stud farms and those declaring not to use separate summer and winter paddocks had significantly shorter ERPs ($p < 0.01$).In conclusion, retained ERPs and no confirmed resistance to IVM were found in Swedish equine establishments practising selective anthelmintic treatment, and supports the use of selective deworming regimens as a means of reducing the risk of anthelmintic resistance development.

1. Introduction

Cyathostominae are ubiquitous to grazing horses worldwide and considered the most common helminth parasite in the horse. Over 50 species and 14 genera of cyathostomins are recognised and individual horses are often co-infected with as many as 15–25 different species (Bellaw and Nielsen, 2020; Chapman et al., 2003; Reinemeyer et al., 1984). Although not considered as pathogenic as the large strongyle, *Strongylus vulgaris*, cyathostomins are associated with the fatal condition of larval cyathostominosis, which can occur when massive numbers of encysted larvae simultaneously emerge from the intestinal mucosa (Lyons et al., 2000; Mair, 1994). In addition, large burdens of cyathostomins can cause weight loss and unthrift in horses, particularly in individuals that are otherwise health-compromised (Love et al., 1999; Murphy and Love, 1997).

Reduced efficacy and the development of resistance to equine

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anthelmintic drugs are emerging concerns and to date, cyathostomin resistance to the benzimidazoles is widespread with increasing number of reports also of pyrantel resistance (Kaplan, 2002; Nielsen et al., 2014; Nielsen, 2022). The anthelmintic group macrocyclic lactones (ML), encompassing ivermectin (IVM) and moxidectin (MOX), became available on the public market in in the 1980 s and is now the most widely used anthelmintic drug class for the treatment of cyathostomins in horses, both in Sweden and in other parts of the world (Elghryani et al., 2019; Hedberg-Alm et al., 2020; Nielsen et al., 2018; Robert et al., 2015; Salle and Cabaret, 2015; Stratford et al., 2014). Official Swedish statistics show that five times as many ML doses were prescribed in 2021 compared with doses of pyrantel and benzimidazole combined (Girma, 2021). However, despite the intense usage of ML, resistance to this drug group has been unexpectedly slow to develop in cyathostomins. Although several reports of suspected resistance in single horses or small populations have been published (Canever et al., 2013; Milillo et al., 2009; Nareaho et al., 2011; Relf et al., 2014; Traversa et al., 2009), confirmed resistance of cyathostomins to IVM with reproducible results was first reported in 2020 (Nielsen et al., 2020). More recently, multi-drug resistance in cyathostomins, including resistance to both IVM and MOX, was demonstrated in the UK (Bull et al., 2023).

A number of techniques to determine the presence of anthelmintic resistance (AR) have been developed. In research settings, so called critical and controlled tests are available, but both require necropsy of the studied animals and are thus not of use for field studies (Nielsen et al., 2022c). *In vitro* tests, such as the egg hatch assay and the larval development assay, lack reference data for susceptible and resistant larvae and with the presence of multiple species in natural cyathostomin infections are prohibitive to practical use (Kaplan, 2002). To date, an *in vivo* method, the faecal egg count reduction test (FECRT), is the most widely used test in field studies to determine AR in horses (Coles et al., 2006; Kaplan, 2002; Nielsen et al., 2022c). This test calculates the percentage of reduction in egg excretion 14 days after anthelmintic treatment and the target efficacy in response to ML treatment is > 99.9% reduction in egg excretion (Kaplan et al., 2023).

The egg reappearance period (ERP), how quickly egg excretion commences post-anthelmintic treatment, has been suggested to be an early indicator of drug resistance (Sangster, 1999), although this has recently been disputed (Nielsen et al., 2022c). Regardless, a shortened ERP reflects a change in response to the applied treatment and shortly after MLs first were introduced, an ERP of at least nine to thirteen weeks after IVM treatment was demonstrated (Boersema et al., 1996; Borgsteede et al., 1993; Demeulenaere et al., 1997). Although the definition of ERP has not been consistent, hindering direct comparisons between studies, these early ERPs for IVM contrast with more recent studies conducted in various countries showing ERPs as short as four weeks (Geurden et al., 2014; Lyons et al., 2008; Molena et al., 2018). In Sweden, however, in a study performed in 2007, an ERP following IVM treatment of at least eight weeks was demonstrated (Osterman Lind et al., 2007). In order to better compare studies, the most recent World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Nielsen et al., 2022c), define ERP as the number of weeks post-treatment for which the upper confidence (or credible) interval (CI) for the mean FECR falls below the mean FECR determined at 14-day post-treatment minus 10%. Hopefully, by using this ERP definition, future studies will render comparable data to better evaluate forthcoming changes in ERP.

The effect of IVM in the Swedish horse population, as determined by the FECRT and ERPs, has not been investigated for the past 15 years. In 2007, prescription-only administration of anthelmintic drugs was implemented in Sweden, with selective anthelmintic treatment henceforth strongly advocated, aiming to slow down the development of anthelmintic resistance. The current efficacy status of IVM in Sweden is, however, not known and therefore, the aims of the present study were to, in selected equine establishments in Sweden, investigate: i) the FECR at 14 days after IVM treatment and ii) the ERPs after IVM treatment.

2. Materials and methods

2.1. Inclusion criteria for participating equestrian establishments

Suitable equestrian establishments were identified through the National Veterinary Institute's (SVA) equine parasite monitoring programme in April-May 2021. In addition, Sweden's three national equestrian centres (Flyinge, Strömsholm and Wången) were contacted and asked for participation. Inclusion criteria for an equestrian establishment to be considered appropriate for the study was a minimum of six horses excreting at least 150 eggs per gram faeces (EPG) at screening before the start of the study and the ability to collect individual faecal samples from each participating horse for a duration of eight weeks.

2.2. Study design

On day 0, a pre-treatment faecal sample was collected from each participating horse. Horses were then treated with a single oral dose of 200 μg/kg IVM paste (Eraquell® 18.7 mg/g), performed by either the horse owner, the veterinarian responsible at the establishment or other responsible personnel. The weight of each horse was estimated using a weight tape and rounded up to the nearest 50 kg to minimize the risk of administrating an inadequate dose of anthelmintic drug. Post-treatment faecal samples were collected at 14 days post- treatment and then at weekly intervals for another six weeks (Fig. 1). All faecal samples were sent by post to the Swedish Agricultural University (SLU) for analysis (see 2.3).

2.3. Faecal analysis

Strongyle FECs were carried out for each horse using a modified centrifugation-enhanced McMaster technique with a theoretical sensitivity of 50 EPG (Coles et al., 1992). Nematode eggs in faecal samples (3 g) were flotated using a saturated NaCl solution with a density of SG= 1.18 g/cm3 (Coles et al., 1992). All samples were performed in duplicates.

2.4. Calculation of faecal egg count reduction and egg reappearance period

Mean FECs and mean faecal egg count reductions (FECRs) with corresponding credible intervals for each establishment before treatment (FECs only) and at 14, 21, 28, 35, 42, 49 and 56 days post-treatment were calculated using the hierarchical Bayesian shiny-eggCounts web interface (Torgerson et al., 2014; Wang et al., 2018). Resistance to IVM was defined as a FECRT with an upper 90% confidence level (UCL) less than an expected efficacy of 99.9%, according to the novel proposed definition by the WAAVP (Kaplan et al., 2023). Farms with FECRTs showing an UCL ≥ 99.9% and a lower confidence level (LCL) of ≥ 96.0% were considered fully susceptible to IVM, rendering a "grey zone" where, if either of these conditions was not met, the result was deemed inconclusive (Kaplan et al., 2023).

ERP was defined as the week in which the upper credible limit of the estimated mean faecal egg count reduction (FECR) fell below the mean FECR measured at two weeks post-treatment minus 10% (Nielsen et al., 2022c). To enable comparison with earlier studies, a second definition for ERP was also used, and defined as the week in which a mean EPG above 100 for all horses in a given establishment was detected.

2.5. Questionnaire

All participating establishments were asked to fill in a questionnaire regarding type of establishment (primary function, total number of horses, estimated number of new horses arriving per year), pasture management, anthelmintic treatment routines and management of new arrivals (anthelmintic treatment, quarantine) (Table 1).

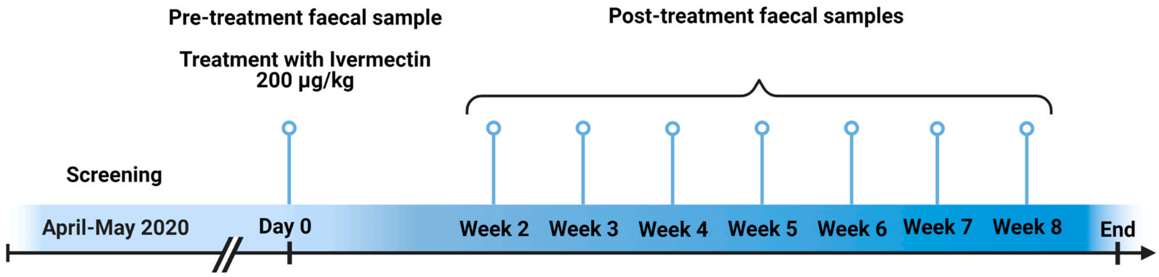

Fig. 1. Experimental design: timeline showing the study design with the timing of treatment and faecal sampling of participating horses.

Table 1
Questionnaire data collected from participating establishments.

Information	Descriptor
Q1: Number of horses*	i) 8–10 ii) 11–20 iii) 21–30 iv) 31–40 v) > 40
Q2: Type of establishment* *	i) livery ii) racing iii) stud iv) riding school v) other
Q3: New arrivals	i) None ii) one/year iii) 2–3/year iv) > 5/year
Q4: Management of new arrivals* *	i) Anthelmintic treatment ii) Anthelmintic treatment after faecal sample iii) no treatment iv) separate box/paddock > 1 week v) separate box/paddock ≤ 1 week vi) no separate box/paddock
Q5: Separate summer/winter paddock	i) yes ii) no
Q6: Faecal removal winter	i) several times/week ii) once/week iii) once/month iv) once/6 months v) once/year vi) never
Q7: Faecal removal summer*	i) several times/week ii) once/week iii) once/month iv) once/6 months v) once/year vi) never
Q8: Harrowing or topping of pasture*, * *	i) summer: once/year ii) summer: twice/year iii) summer: > twice/year iv) winter: once/year v) winter: twice/year vi) winter: > twice/year vii) never
Q9: Other pasture management	Free text
Q10: Anthelmintic routine* *	i) treatment only if indicated by faecal sample ii) use of FECs and diagnostics for *S. vulgaris* at least once/year iii) routine deworming once/year iv) routine deworming 2–4 times/year
Q11: Anthelmintic drug (s) used past two years* *	i) FBZ[a] ii) PYR[b] iii) ML[c] iv) ML + PRZQ[d] v) no recollection vi) other

*To avoid singularity issues, the questions were recoded for statistical analysis: Q1: ≤ 20 or ≥ 21 horses; Q7: yes or no faecal removal; Q8: yes or no harrowing or topping.
* * More than one alternative possible.
[a] fenbendazole, [b] pyrantel, [c] macrocyclic lactone, [d] macrocyclic lactone and praziquantel combination.

2.6. Statistical analysis

Possible associations between the questionnaire answers and the ERP (def.1, Table 2) were evaluated using the package xgboost v.1.6.0.1 (Chen and Guestrin, 2016) that implements linear model solver and tree learning algorithms in R v4.2.2 (Team, 2022). Thereafter, the factors most strongly associated with the ERP were evaluated using generalized linear models (GLM) with quasipoisson error distribution to avoid over-dispersion. The significance was set at the 0.05 level and non-significant factors were removed stepwise starting backwards from the factors that showed the least association in the calculation above, until only significant terms remained. Factors affecting the time to when strongyle eggs could first be detected in the faeces post-treatment were calculated on an individual horse basis, and, hence, these values were combined with the questionnaire data to form a repeated measure dataset. For this combined dataset, importance was calculated as described above. To test for significance, a generalized linear mixed model was implemented with establishment as a random factor and again, non-significant terms dropped stepwise following the same pattern as described above. The package ggplot2 (Wickham, 2016) was used for all statistical visualizations.

3. Results

3.1. Demographic data, faecal egg counts and faecal eggs counted

Sixteen equestrian establishments with a total of 134 horses participated in the study. A variety of breeds were represented, namely Warmbloods (46%), pony breeds (22%), Standardbreds (11%), cold bloods (8%), Thoroughbreds (7%) and Icelandic horses (4%), with two horses of unknown breed. For a description of each establishment (establishment type, total number of horses and their age span), see Table 2. The equestrian establishments were spread over a large geographical area of Sweden (Fig. 2).

The mean EPG pre-treatment varied from 399 to 2789 between the establishments, with the lowest and highest EPGs detected pre-treatment being 150 and 5600, respectively. The mean number of actual strongyle eggs counted per horse at each establishment ranged from 17 to 125 with a mean and median of 40 and 31 faecal eggs, respectively. The total number of actual faecal eggs counted per establishment ranged from 116 to 1128 eggs, with a mean of 339 total eggs and median of 268 total eggs.

3.2. FECRT and ERP

Fourteen of the establishments (87.5%) fulfilled the set criteria for full susceptibility of cyathostomins to IVM treatment, with UCLs ≥ 99.9% and LCLs ≥ 96.0%. The remaining two establishments had FECRs rendering inconclusive results, with LCLs < 96.0%, but no farm was defined as resistant (UCL < 99.9%). The ERP, defined as the week in which the upper credible limit of the estimated mean FECR fell below the mean FECR measured at two weeks post-treatment minus 10%, occurred from six weeks to over eight weeks in all establishment (def. 1, Table 2). A second definition, with ERP defined as the week in which the mean EPG exceeded 100, was also included, for comparative reasons (def. 2, Table 2). Using this latter definition, two establishments (numbers 3 and 6) had an ERP of five weeks. The remaining establishments had an ERP, according to this second definition, of six to over eight weeks. Dividing the entire horse population (all establishments) into age groups showed an increase in ERP (both definitions) with increasing age (Table 3), and at an individual level, there was a significant association between age and the week in which strongyle eggs were first detected, with young individuals excreting strongyle eggs earlier after treatment compared with older horses ($R = 0.21$, $p = 0.015$) (Fig. 3 A). In addition, young horses had significantly higher EPG levels prior treatment compared with older horses, with a negative correlation between age and pre-treatment EPG-level ($R = -0.37$, $p < 0.001$), and, consequently, there was also a significant negative correlation between pre-treatment EPG level and the week in which strongyle eggs were detected post-treatment ($R = -0.38$, $p < 0.001$) (Fig. 3 B, C). Furthermore, there was a significant association between type of establishment and ERP (def. 1), with riding schools and stud

Table 2
Selected equestrian establishments, specified by type of establishment and number and age of participating horses, with each establishments' calculated FECR and ERP. ERP was defined as the week in which the upper credible limit of the estimated mean FECR fell below the mean FECR at two weeks post treatment minus 10% (def. 1) or the week in which the mean EPG exceeded 100 (def. 2). The ERP threshold was set to when the upper confidence (or credible) interval (CI) for the mean FECR fell below the mean FECR determined at 14-day post-treatment minus 10%.

Farm no.	Type of establishment	Participating horses (total horses at establishment)	Age (mean; median; range)	Mean (range) EPG pre-treatment	Total number of strongyle eggs counted	Mean FECR (95% CI) at two weeks post-treatment	ERP threshold	ERP (def. 1)	ERP (def.2)
1	Livery stable, riding school	14 (> 40)	12.8; 12; 7–29	931 (250–2700)	532	99.2% (97.6–99.9)	89.2%	8 weeks	7 weeks
2	Stud farm, national equestrian centre	9 (> 40)	10; 9; 6–14	616 (150–1200)	236	99.8% (97.5–100)	89.8%	8 weeks	8 weeks
3	Stud farm	9 (31–40)	3.2; 3; 2–5	1393 (700–3500)	540	99.9% (99–100)	89.9%	6 weeks	5 weeks
4	Riding school	10 (31–40)	11.5. 11.5. 8–16	777 (500–950)	318	99.9% (98.1–100)	89.9%	8 weeks	8weeks
5	Livery stable	8 (11–20)	8.1; 5; 2–24	709 (150–1550)	240	99.8% (97.4–100)	89.8%	8 weeks	8 weeks
6	Stud farm	9 (> 40)	1; 1; 1–1	2789 (850–5600)	1128	> 99.9% (99.5–100)	89.9%	7 weeks	5 weeks
7	Livery stable, riding school	8 (21–30)	13.6; 14.5; 8–17	834 (150–1750)	286	99.9% (97.8–100)	89.9%	8 weeks	8 weeks
8	Livery stable, sales stable	9 (31–40)	8.9; 10; 3–16	737 (150–1450)	268	99.8% (97.7–100)	89.8%	> 8 weeks	> 8 weeks
9	Livery stable, riding school, national equestrian centre	7 (> 40)	5.6; 4; 3–10	744 (350–1650)	224	99.8% (97.3–100)	89.8%	7 weeks	6 weeks
10	Livery stable	7 (11–20)	9.3; 8; 4–24	399 (150–650)	116	99.6% (94.6–100)	89.6%	> 8 weeks	> 8 weeks
11	Livery stable	6 (31–40)	11.8; 13; 5–17	676 (200–1350)	172	99.7% (96.4–100)	89.7%	> 8 weeks	> 8 weeks
12	Livery stable	8 (11–20)	10.5; 9.5; 6–20	540 (150–1150)	182	99.8% (96.5–100)	89.8%	8 weeks	8 weeks
13	Livery stable, racing stable	6 (8–10)	4.3; 2.5; 1–10	1230 (500–3500)	326	99.9% (98.1–100)	89.9%	> 8 weeks	> 8 weeks
14	National equestrian centre	7 (> 40)	6.4; 6; 2–13	1371 (550–4300)	438	99.9% (98.6–100)	89.9%	> 8 weeks	8 weeks
15	Livery stable	10 (8–10)	12.8; 12.5; 5–24	650 (150–1200)	268	99.8% (97.6–100)	89.8%	8 weeks	8 weeks
16	Livery stable	7 (> 40)	12.7; 12; 3–21	498 (150–1000)	144	99.7% (95.7–100)	89.7%	8 weeks	> 8 weeks

farms showing significantly shorter ERPs compared with other types of equestrian establishments ($p < 0.01$). The total number of horses at the establishment did not affect ERP.

3.3. Questionnaire: pasture management

The majority of establishments used separate summer and winter paddocks (81%). However, faecal removal was less commonly used in the summer as opposed to winter months. Harrowing or topping of the pasture was used by the majority of respondents during the summer months (75%), most often on a yearly basis. The results of the questionnaire regarding harrowing/topping and faecal removal are shown in Fig. 4. A quarter (25%) of the establishments used other pasture management methods, most often co-grazing or alternate grazing with a ruminant species, with one responder declaring to rest pastures regularly. Establishments reporting not to use separate summer and winter paddocks had significantly shorter ERPs compared with establishments that did utilise separate grazing areas ($p < 0.01$). No other pasture management factor showed a significant association with ERP.

3.4. Questionnaire: anthelmintic routines

The results of the questionnaire regarding anthelmintic routines and management of new horses are summarised in Table 4. Fifty percent of establishments had more than five new horses arrive each year and the majority of respondents reported that all new horses were treated with an anthelmintic drug at arrival (63%). However, only a minority of the establishments (31%) reported the use of separate areas for the new horses for a duration of longer than one week after arrival. The questionnaire did not specifically ask if efficacy testing after anthelmintic treatment was used prior to introducing new arrivals to the resident horses, but was deduced not to be employed by the majority of owners, since they reported to quarantine new horses for a week or less (69%). The vast majority (88%) reported the use of faecal egg counts (FECs) and extended diagnostics for *S. vulgaris* on a yearly basis, with only one establishment declaring to use anthelmintic drugs 2–4 times per year, regardless of diagnostics. Macrocyclic lactones had been used by all establishments within the last two years, and as a sole drug in 25% of establishments. The remaining 75% of establishments had used MLs together with varying combinations of other anthelmintic drugs. There was no statistical association between the management of new arrivals, anthelmintic routine or drug most commonly used and the establishment's ERP.

4. Discussion

The present study showed no evidence of drug resistance in cyathostomins to IVM, with UCLs of the FECRs at 14 days post-treatment equal to or greater than 99.9% in all participating equine establishments. In addition, full susceptibility in cyathostomins to IVM could be verified in 14 out of 16 farms (Kaplan et al., 2023) Further, the ERPs post-IVM treatment were at least six weeks in all horse populations studied, with a majority (81%) demonstrating an ERP of at least eight weeks. Our results tentatively support the use of selective anthelmintic

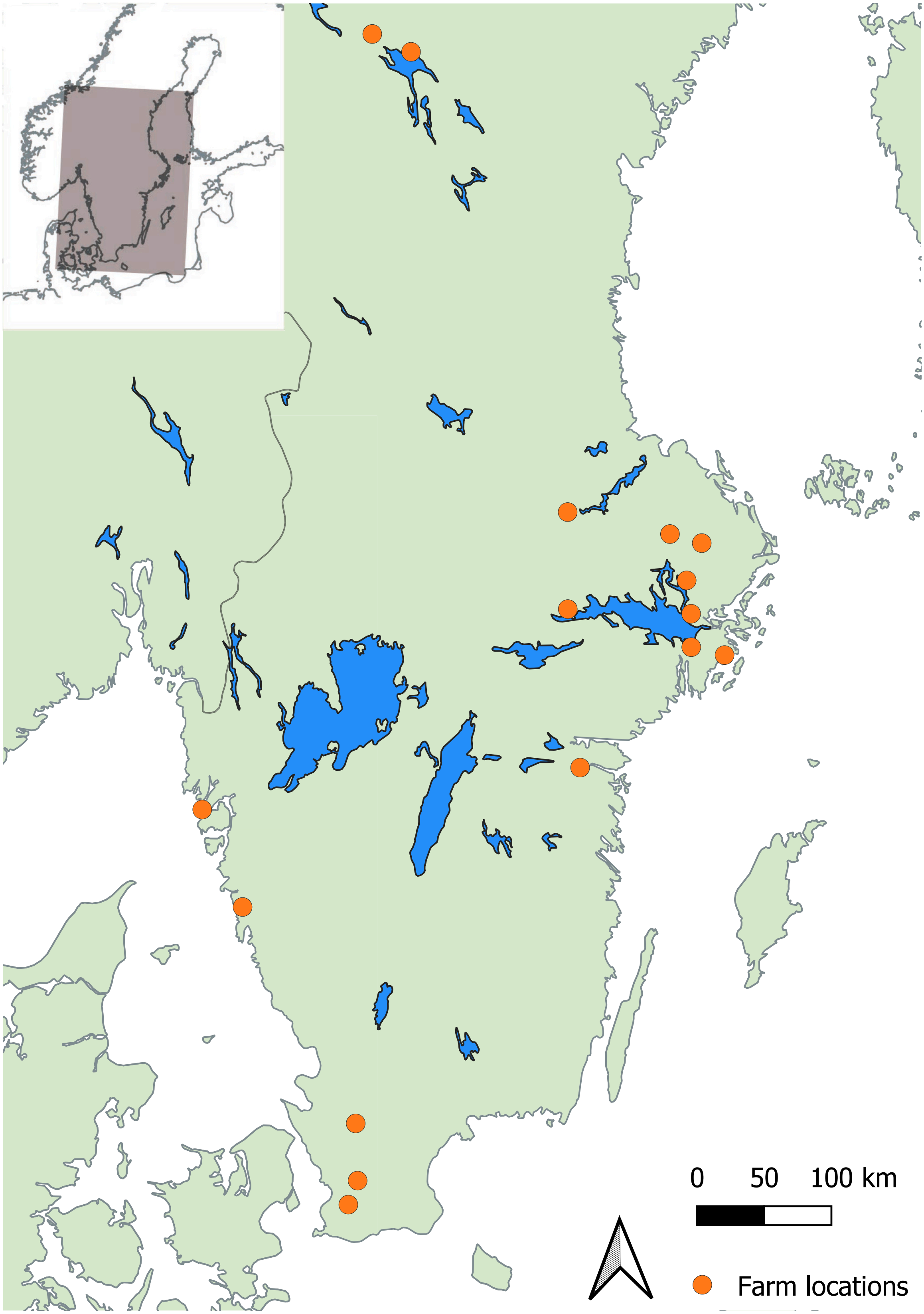

Fig. 2. Geographical distribution of the participating equestrian establishments.

Table 3
FECR and ERP in three different age categories. ERP was defined as the week in which the upper credible limit of the estimated mean FECR fell below the mean FECR at two weeks post treatment minus 10% (def. 1) or the week in which the mean EPG exceeded 100 (def. 2).

Age span	Number of horses	Mean (range) EPG pre-treatment	Mean FECR (CI) 14 days post-treatment	ERP (def. 1)	ERP (def. 2)
1–2 years	17	2084 (500–5600)	> 99.9% (99.6–100)	5 weeks	5 weeks
3–4 years	19	1184 (350–4300)	> 99.9% (99.3–100)	8 weeks	7 weeks
> 4 years	98	765 (150–3500)	99.9% (99.6–100)	8 weeks	8 weeks

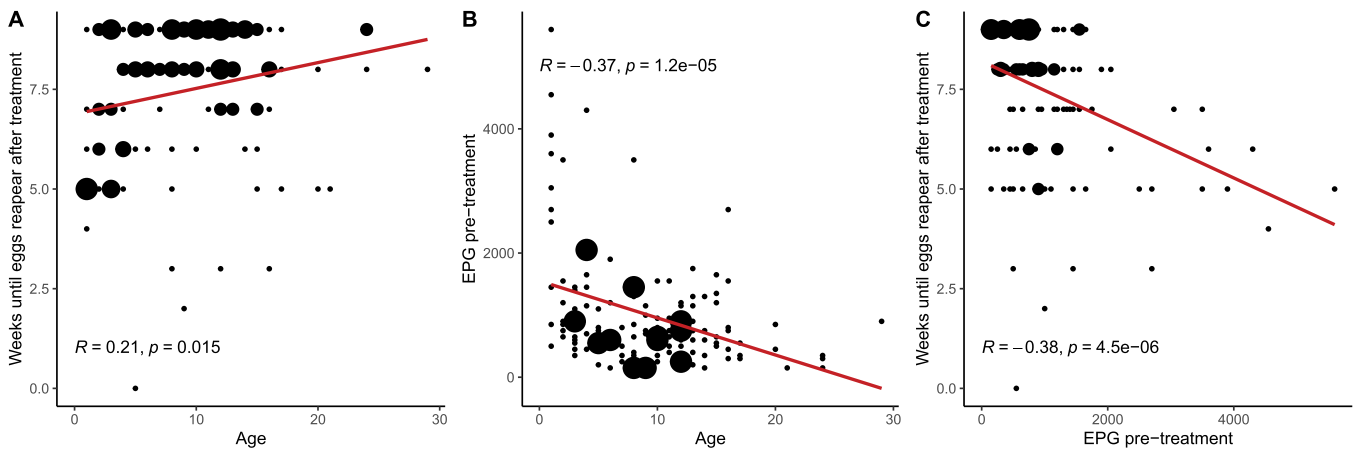

Fig. 3. Correlation plots at an individual level depicting: A. the correlation between age and the week in which small strongyle eggs reappeared in the faeces, B. the correlation between age and the EPG pre-treatment level, C. the correlation between the EPG pre-treatment level and the week in which strongyle eggs reappeared in faeces.

treatment as a means to slow down the development of anthelmintic resistance in the horse population and agree with simulations that have shown selective therapy regimens to delay the development of resistance, particularly in colder climates (Geurden et al., 2021; Nielsen et al., 2019).

In order to adhere to the most current standardization when performing FECRTs, the present study utilised the new WAAVP guidelines to classify the participating farms as a result of the calculated FECRTs (Kaplan et al., 2023). However, the study was unable to meet the set criteria for total number of counted strongyle eggs in all farms, and two out of 16 farms rendered inconclusive results based on this new and current classification system (Kaplan et al., 2023). Nonetheless, the remaining 14 farms all demonstrated full susceptibility of cyathostomins to IVM. Furthermore, the study showed sustained ERPs after IVM treatment, which has substantial practical implications regarding pasture parasite infection pressures and larval burdens, as recently demonstrated using simulation tests, and further support maintained efficacy of IVM in the studied equine population (Nielsen et al., 2023).

Even though MLs are reported to be the most commonly used drug class at Swedish horse facilities, (Hedberg-Alm et al., 2020; Tyden et al., 2019), there is an overall conservative use of anthelmintic drugs in this country, with a majority of equine owners exclusively treating horses based on faecal diagnostics (Hedberg-Alm et al., 2020). Only a minority of Swedish horses are treated up to four times per year regardless of diagnostics, with a mere 6% of horse owners (i.e. one establishment) in the present study and between 13% and 20% in a previous questionnaire survey declaring to do so (Hedberg-Alm et al., 2020). This contrasts with other countries, such as the USA, the United Kingdom (UK) and Ireland, where more intense anthelmintic drug treatment regimens appear to be common (Elghryani et al., 2019; Rendle et al., 2021; Robert et al., 2015). For example, most Kentucky Thoroughbred yearling farms perform routine regular anthelmintic treatments at least six times per year (Robert et al., 2015). In the UK, there are indications that only one FEC is performed for every eleven doses of anthelmintic drug sold (Rendle et al., 2021), despite over 60% of respondents in two surveys declaring to use regular FEC monitoring (Stratford et al., 2014; Tzelos et al., 2019). Furthermore, a recent survey conducted in Ireland showed that the majority of farms (81%) treated horses 4–5 times per year, in most cases without prior diagnostic testing (Elghryani et al., 2019). Reports indicative of drug resistance to MLs are now appearing, with FECRs at 14 days post-treatment of below 95% demonstrated in several countries such as the USA, the UK, Finland, Italy, Brazil and Australia (Abbas et al., 2021; Canever et al., 2013; Flores et al., 2020; Geurden et al., 2014; Milillo et al., 2009; Nareaho et al., 2011; Nielsen et al., 2022a; Relf et al., 2014; Toscan et al., 2012). In addition, definite proof of resistance to IVM with reproducible results in a large group of horses was demonstrated in imported Irish horses in 2020 (Nielsen et al., 2020) and resistance to both IVM and MOX was recently documented in a UK yearling farm (Bull et al., 2023). Moreover, in recent years, a growing number of studies have reported a decrease in ERPs for IVM in numerous countries, such as Italy, Belgium, the Netherlands, the UK, Germany and the USA (Geurden et al., 2014; Lyons et al., 2008; Molena et al., 2018; von Samson-Himmelstjerna et al., 2007). Although a reduced ERP may not be a sign of true anthelmintic resistance, as originally postulated, it nonetheless demonstrates an undesired selection towards cyathostomins that more rapidly resume egg production post-treatment (Nielsen, 2022). Thus, several equine populations in various countries practising routine interval treatment without frequent use of diagnostics are showing signs of reduced efficacy of ML against cyathostomins.

The World Association for the Advancement of Veterinary Parasitology (WAAVP) (Nielsen et al., 2022c) has recently advocated the use of a sole definition for calculating the ERP to better compare future studies. A recent review revealed that eight different definitions for the ERP have been used over the past 30 years, such as the week in which the mean EPG exceeds 100 (Macdonald et al., 2023). In 2007, ERPs calculated using this definition demonstrated ERPs of eight to ten weeks in Swedish horse farms (Osterman Lind et al., 2007). Therefore, for comparative reasons, we also calculated the ERP according to this definition in the

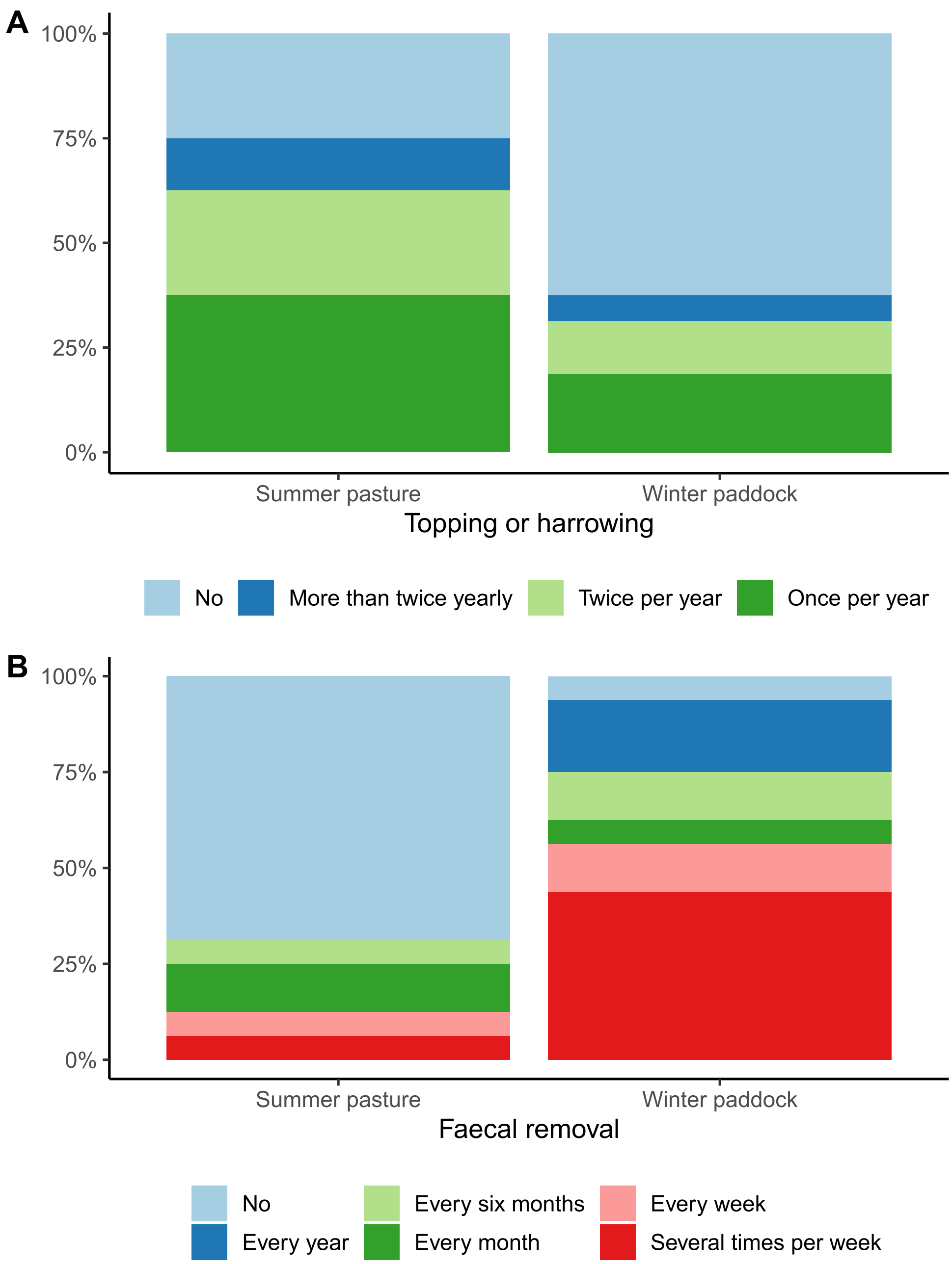

Fig. 4. Pasture management methods employed by the included equestrian establishments as assessed by questionnaire data: A. Frequency of use of harrowing or topping in the summer pasture and winter paddocks. B. Frequency of use of faecal removal in the summer pasture and winter paddocks.

present study, which rendered somewhat shorter ERPs following IVM treatment, from five to over eight weeks, although a majority of establishments still showed ERPs of at least eight weeks (75%). The most likely explanation for the shorter ERPs in the present study is, however, not a reduced efficacy, but a difference in the ages of the horses studied, with the mean ages of the horses in the establishments with the lowest ERPs in the present study being only 1.0 and 3.2 years, and lower than those of the horses in Osterman-Lind's study (6.0 and 9.0 years). For cyathostomins, FECs, and also ERPs, are age-dependent and for comparative reasons, the mean age of the horses needs to be similar for definitive conclusions to be drawn (Nielsen, 2022). Although not a consistent finding, shorter ERPs in young horses have been shown in several previous publications (Demeulenaere et al., 1997; Eysker et al., 2008; Herd and Gabel, 1990). Accordingly, in the present study, division of the horses into age groups showed an increase in ERP with increasing age using both ERP definitions. Further, the present study showed that, at an individual level, strongyle egg excretion in young horses occurred significantly earlier compared with older individuals. Nonetheless, the ERPs demonstrated in young horses in the present study are lower than those reported in the 1990 s, when ERPs even in yearlings were reported to be at least nine weeks (Boersema et al., 1996; Borgsteede et al., 1993). Thus, even though our results do not indicate a reduced efficacy of IVM compared with the effect 15 years ago, egg excretion is detected sooner than originally presented for IVM also in the Swedish horse population.

In the present study, there was a significant association between type of establishment and ERP, with riding schools and stud farms showing shorter ERPs compared with other types of establishments. Stud farms will have a wide range of ages present, including foals and yearlings, and with young individuals showing shorter ERPs than their older counterparts, this is a probable reason for the shorter ERPs demonstrated in this

Table 4
Strategies employed for new arrivals and general anthelmintic treatment routines at the participating establishments.

Question	Response alternatives	Response (%)
Q1: Average number of new horses/year	> 5	50%
	2–3	44%
	1	6%
Q4: Anthelmintic treatment of new horses	Yes	63%
	No	6%
	Based on faecal sample	31%
Q4: Quarantine of new horses*	Separate box/paddock > 1 week	31%
	Separate box/paddock ≤ 1 week	38%
	No quarantine	6%
Q10: Anthelmintic routine (more than one option possible)	FEC and diagnostics *S. vulgaris* at least once/year	88%
	Anthelmintic treatment only if indicated by faecal sample	69%
	Anthelmintic treatment once per year regardless diagnostics	0%
	Anthelmintic treatment 2–4 times/year regardless diagnostics	6%
Q11: Anthelmintic drug used past two years	ML[a]	25%
	ML[a], PYR[b], BZ[c], ML[a] + PRZQ[d]	19%
	ML[a], ML[a] + PRZQ[d]	19%
	ML[a], BZ, ML[a] + PRZQ[d]	13%
	ML[a], PYR[b]	6%
	ML[a], PYR[b], ML[a] + PRZQ[d]	6%
	ML[a], PYR[b], BZ[c]	6%
	ML[a], BZ[c]	6%

[a] macrocyclic lactone, [b]pyrantel , [c]benzimidazole, [d]praziquantel; * one farm chose all options

establishment type. The reason for the association between riding schools and ERP is less clear. Regarding pasture management, establishments not providing different paddocks by season showed shorter ERPs. Parasite eggs accumulate during the summer and reach a peak in late autumn (Corning, 2009), and thus it is possible that the use of permanent pastures resulted in higher parasite infection levels in the residing horses, requiring more frequent anthelmintic treatments and a greater selection pressure for resistance (Geurden et al., 2021; Nielsen et al., 2019). However, further studies confirming this association are needed to more clearly elucidate what impact pasture management methods may have on anthelmintic treatment frequency and, consequently, the risk of reduced drug efficacy.

In conclusion, the present study showed that in Swedish equine establishments practising selective anthelmintic treatment regimens, no resistance to IVM was demonstrated, with all but two establishments displaying full drug susceptibility. Further, ERPs following IVM treatment were at least eight weeks in the vast majority of farms. The results thus appear to support the use of selective deworming strategies in horses as a means to reduce the risk of anthelmintic resistance development.

Institutional Review Board Statement

Ethical review and approval were waived for this study in accordance with relevant guidelines and regulations issued by the Swedish Board of Agriculture's regulations and general advice on laboratory animals (SJVFS 2019:9, case no. L150).

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Osterman-Lind Eva: Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Martin Frida:** Writing – review & editing, Visualization, Software, Formal analysis. **Lindfors Rebecca:** Project administration, Investigation. **Roepstorff Nina:** Project administration, Investigation. **Tydén Eva:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Hedberg Alm Ylva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Funding acquisition, Formal analysis, Data curation. **Hedenström Ulf:** Project administration, Investigation. **Fredricson Isabelle:** Project administration, Investigation. **Halvarsson Peter:** Writing – review & editing, Visualization, Software, Formal analysis.

Declaration of Competing Interest

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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.2023.110007.

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