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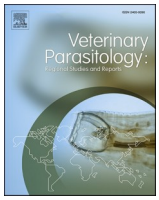

Original Article

Egg reappearance periods associated with anthelmintic treatments given to horses in winter and summer over two years

Ian Scott *, Kevin E. Lawrence, Erica K. Gee

Tāwharau Ora - School of Veterinary Science, Massey University, Private Bag 11222, Palmerston North 4410, New Zealand

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ABSTRACT

Shortened egg reappearance periods (ERP) have been seen as an early warning of anthelmintic resistance development in cyathostomins in horses. The hypothesis has been that in these instances, efficacy against egg laying adult cyathostomins remains high, but a decline in activity against later larval stages leads to the earlier resumption of egg shedding after treatment. In this study using a single herd of horses we investigated the ERP of a number of commonly used equine anthelmintics and examined whether ERP might show seasonal variation between winter and summer. Four main Faecal egg count reduction tests (FECRT1–4) were conducted respectively in Winter (Jun/Jul) 2019, Summer (Jan/Feb) 2020, Winter 2020 and Summer 2021. The tests examined the efficacy and ERP of ivermectin, moxidectin, abamectin and fenbendazole. Egg counts of two groups of horses were monitored before and for 6–7 weeks after treatment - however long it took for counts to return to at least 10 % of what they had been before treatment. One additional FECRT was also conducted, using a second abamectin-containing product (FECRT5 - Spring 2020). Treatment with ivermectin (FECRT1–4), moxidectin (FECRT 1–2) and the first abamectin product tested (FECRT3) all reduced egg counts by >99 % for 4 weeks after treatment, with ERP of 5–7 weeks and with minimal differences between the 3 treatments. There was a tendency for counts to rise more rapidly in summer, and in the second year of testing as opposed to the first. Both the second abamectin product (FECRT5) and the fenbendazole (FECRT4) were found to be ineffective, reducing egg counts immediately after treatment by 68 and 52 % respectively.

1. Introduction

The small strongyles, also known as the cyathostomins, are now seen as the major parasites of horses, representing close to 100 % of the roundworm parasites present in horses, other than foals, kept at pasture in many countries (Kaplan and Nielsen, 2010) including Aotearoa New Zealand. Anthelmintic efficacy in equines is most commonly judged by examining how much egg counts decline after treatment and efficacy is thus being assessed only against the egg laying adult parasites. When an adequate reduction of egg counts occurs, it is usually taken as evidence that resistance is not present, yet, even though anthelmintics such as those in the macrocyclic lactone (ML) class can still reduce cyathostomin egg counts to zero or near-zero after treatment, the amount of time required for egg shedding to return, the egg reappearance period (ERP), is often now shorter than it used to be (MacDonald et al., 2023). The traditional argument has been that shortened ERP have arisen as efficacy of ML has declined against larval stages, particularly later L4 present in the gut lumen at the time of treatment, whilst obviously still remaining high against the egg laying adults, although an alternative hypothesis has been proposed based on accelerated larval development (Nielsen et al., 2022a; MacDonald et al., 2023).

Thus far there have been no confirmed and peer-reviewed reports of overt ML-resistance in cyathostomins in Aotearoa New Zealand although there have been increasing reports overseas (Flores et al., 2020; Abbas et al., 2021, 2024; Lignon et al., 2021; Martins et al., 2021; Merlin et al., 2024; Nielsen et al., 2020, 2022b). Morris et al. (2019) found no evidence of ML-resistance in strongyle (presumed to be cyathostomin) egg count reductions in their 2011 study conducted across multiple farms in Aotearoa New Zealand. Likewise, in another local study, carried out across six Waikato stud farms (Rosanowski et al., 2017), the efficacy of ivermectin against cyathostomins was determined to be 100 % for the vast majority of treated yearling horses, with no eggs detected for at least two weeks for 113 out of 117 animals evaluated. There was, however, a shortened egg reappearance period (ERP) reported in three of the four farms for which data could be obtained.

Many studies have now shown the existence of shortened ERP, but

* Corresponding author.
E-mail address: I.Scott@massey.ac.nz (I. Scott).

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few if any studies have looked at how repeatable ERP are, and whether there is any seasonal influence on ERP. In one study there was a difference in the pharmacokinetics of ivermectin when horses were treated in spring or autumn (Sokol et al., 2015), with higher blood concentrations being achieved in the first four hours after treatment in spring. Although this study did not assess ERP there may have been some difference with higher egg counts 75 days after treatment in the spring.

Further to any pharmacological differences between seasons, is the potential for population differences between seasons. Horses are fairly unique amongst domestic animal species in that adult nematode parasites typically make up a small minority of the total worm burden present, and this declines as a percentage in winter. In winter, more larval stages are present, principally since many early L3 stages become inhibited in development at this time, with the adult population representing as little as 1–3 % of the total burden (Bairden et al., 2001, 2006), but it is also possible that other larval stages might be slower to develop at this time. Although somewhat unlikely, if it were to occur then this could also account for ERP differences between seasons.

The study reported here was designed to examine the efficacy and ERP of three commonly used ML anthelmintics of horses – ivermectin, moxidectin and abamectin - and one anthelmintic in the benzimidazole class – fenbendazole. The products were tested in four main FECRT conducted in winter and summer over the course of 2 years, with only ivermectin used in all 4 tests. In addition, the opportunity was taken to also examine the efficacy of a second, abamectin containing product, that had been the product used as the main anthelmintic in the herd prior to this study, but its efficacy never established.

2. Materials and methods

2.1. Animals

A total of 26 adult horses, both male and female, were used in the study over the two years, representing the entirety of the Massey University School of Veterinary Science teaching herd, except one teaser stallion managed separately. One horse was an ex-Kaimanawa horse, the Kaimanawas being a small feral population of horses in Aotearoa New Zealand, whose exact age is unknown, but it had been with the herd for a number of years. The remaining horses were all Standardbreds or Thoroughbreds, between 8 and 22 years of age. The horses were maintained in 3 separate grazing mobs and kept in these groups throughout the study period. Grazing history was such that (parasite) differences between mobs were not expected.

Prior to this study, the horses were only being treated with anthelmintic if their egg counts exceeded a trigger level of 200 eggs per gram of faeces, but this was not being rigorously applied. The last time any horses had been treated was at the beginning of April 2019, when 2 horses were treated. Before that 3 had been treated in March, and 11 in February. The remaining animals had not been treated in the 6 months prior to the study, with some not having been treated for more than a year.

2.2. Faecal egg count reduction tests

The same horses, with minor additions and losses over time, were used in the four main faecal egg count reduction tests (FECRT 1 to 4) conducted consecutively in winter then summer over two years. The start dates (Day 0) were as follows: FECRT1, 27 June 2019 (Winter); FECRT2, 9th January 2020 (Summer); FECRT3, 11th June 2020 and FECRT4, 14th January 2021. The FECRT used a fairly standard format of sampling the same animals before and after treatment (Nielsen et al., 2013) with weekly monitoring to determine ERP.

Prior to FECRT1, the horses were randomly divided into two groups after stratification based on initial egg counts (21 June 2019) and grazing mob. The aim was for 12 horses in each group for each test, but due to some horses having zero counts on Day 0 this was never possible. Across the 4 tests, 1, 3, 1 and 5 horses were excluded from analyses due to having zero counts on the day of treatment. In addition, 3 horses (1 group 1, 2 group 2) were removed from the herd over time for reasons unrelated to the study, and only 2 were replaced.

The average ages of the 2 groups at the start of the study were 14.8 and 14.9 years, with 7 female horses in group 1 and 6 in group 2, the rest being male (geldings). At the start of the study, Group 1 consisted of 3, 5 and 4 horses from the 3 grazing mobs, whilst for Group 2 the corresponding numbers were 4, 4 and 4.

In all 4 main tests (FECRT1 to 4), the same horses (Group 1) received ivermectin at a minimum dose of 200 μg/kg (Equimax LV®, Virbac New Zealand Ltd), whilst the horses in Group 2 received moxidectin (400 μg/kg - Equest Plus Tape®, Zoetis New Zealand Ltd. - FECRT 1,2) and then abamectin (200 μg/kg - Genesis Horse Wormer®, Boehringer Ingelheim Animal Health NZ - FECRT3) and finally fenbendazole (7.5 mg/kg - Panacur 100®, MSD Animal Health - FECRT4). The number of horses in each group in FECRT1 to 4, the anthelmintic treatments, year, and season are summarized in Table 1.

The additional test (FECRT 5) for the efficacy of the second abamectin containing product (200 μg/kg - Farnam MecWorma and Bot Broad Spectrum Worm Paste for Horses®, International Animal Health Products Ltd) was conducted approximately 2 weeks after the completion of FECRT3 (Day 0, 13th August 2020). In this test, egg counts were performed on the day of treatment and 7 ($n = 9$) and 15 ($n = 3$) days after treatment. The reason for this test was principally to assess the efficacy of the product that had been used as the main anthelmintic given to the herd in the period leading up to the study. This additional test used a mixture of the 12 animals with highest counts from both groups 1 ($n = 7$) and 2 ($n = 5$).

All products used (except Panacur 100®) were registered paste formulations delivered orally by graduated syringe and all (except Farnam MecWorma and Bot Broad Spectrum Worm Paste for Horses®) included praziquantel (2.5 mg/kg minimum dose - except for Equimax LV, 1.5 mg/kg). Fenbendazole liquid was administered pre-mixed in proprietary grain-based horse feed. One Group 2 animal refused to eat the feed and was dosed with ivermectin instead, switching groups. In treating the horses, all received a dose volume rounded up to the next 50 kg representing the next notch on the syringe, i.e. horses between 501 and 549 kg would be given doses sufficient for 550 kg bodyweight. The horses weighed on average 540, 547, 522 and 562 kg at the start of FECRT 1 to 4 respectively.

Faecal samples were collected weekly, from 1 week before treatment, on the day of treatment and then for up to 7 weeks thereafter. Faecal sampling continued until the ERP, as defined later (section 2.4), had been achieved. Faecal samples were removed directly from the rectum of each animal or picked up off a clean concrete floor immediately after deposition.

2.3. Parasitology

Egg counts were done using 2 g of faeces in a modified McMaster method as described by Stafford et al. (1994) but with a 3-chambered McMaster slide such that each egg counted represented a count of 16.7 eggs per gram (in practice this figure was rounded up to 17). Larval cultures were prepared using faeces not used for egg counts. Faeces was mixed with water and vermiculite and kept at 20 °C for approximately 14 days to allow development to the third larval stage for later identification based on published morphological descriptions (Russell, 1948).

2.4. Calculation of efficacy and determination of ERP

Efficacy was evaluated by expressing the eggs present in the weeks after treatment as a percentage of those present on Day 0 according to the following formula:

Table 1
Summary of when, year and season, the main FECRT, 1 to 4, were conducted, and which anthelmintic drugs were used. Also shown is the number of horses used in each test, the number treated and the number with a positive egg count on Day 0.

Year	FECRT	Season	Group 1			Group 2			Total Treated/ Positive
			Treated (n)	Positive (n)	Anthelmintic	Treated (n)	Positive (n)	Anthelmintic	
2019	1	Winter[1]	12	12	Ivermectin	12	11	Moxidectin	24/23
2020	2	Summer[2]	12	11	Ivermectin	12	10	Moxidectin	24/21
2020	3	Winter[2]	12	11	Ivermectin	11	11	Abamectin	23/22
2021	4	Summer[2]	13*	11	Ivermectin	10	7	Fenbendazole	23/18

[1] Follow up period 7 weeks, [2]Follow up period 6 weeks.
* One group 2 animal refused its treatment so was transferred to Group 1.

$$\text{Efficacy } (\%) = \frac{(\text{pre} - \text{treatment count} - \text{post} - \text{treatment count})}{\text{pre} - \text{treatment count}} \text{ x } 100$$

The egg reappearance period for each drug was assessed by three methods. The first two methods simply recorded the first week when egg counts had risen to 10 % or more of the pre-treatment values, using both geometric mean (GM) counts (Method 1) and arithmetic means (AM – Method 2), equivalent to when efficacy had declined to less than 90 %. Calculation of geometric means, more appropriately termed Williams' means (Alexander, 2012), required the addition and later subtraction of a bias correction term (BCT) to all values (Falzon et al., 2015). In this case the BCT was set at half the minimal detectable egg count, i.e. 8.3.

This study was conducted before the publication of the updated guidelines for testing anthelmintic efficacy in equines (Nielsen et al., 2022c) which established a definition of the ERP as the time taken for the upper confidence limit of the reduction of egg counts to fall below the initial reduction observed two weeks after treatment minus 10 %. This criterion was applied to the study data as Method 3 and the upper 95 % confidence limits (credible intervals) for the reductions were calculated using a Bayesian hierarchical model via an online interface (http://shiny.math.uzh.ch/user/furrer/shiny-eggCounts/). The model specified paired counts with 17 as the correction or multiplication factor. The model calculated upper and lower 95 % CI and also the probability that efficacy was greater than 90 %.

2.5. Statistical analyses

Initial Day 0 egg counts (log10 transformed) were compared for Groups 1 and 2 across FECRT 1 to 4 using one-way analyses of variance using GraphPad Prism Version 8. For each group, the pre-treatment counts for one log-transformed FECRT test failed the Shapiro Wilk test of normality whilst passing the equivalent Kolmogorov-Smirnov test. This likely reflects the small sample sizes and although less than satisfactory we proceeded with our statistical analysis of log-transformed data. Both Bartlett's and Browne-Forsythe tests confirmed homogeneity of variances.

2.6. Survival analysis

All data entries for horses where the day zero FEC was zero were removed from the analysis. A decision was made to analyse the data using survival analysis. Survival analysis is a branch of statistics concerned with modelling the time it takes for a certain event to occur. Often that event may be medical, such as death from a specific disease, or mechanical, such as failure of a particular structure. In this case the event was egg reappearance and the time to egg reappearance, referred to as the egg reappearance period (ERP), was calculated for each individual horse as the time in weeks until the first post-treatment FEC was greater than 10 % of the pre-treatment FEC, recorded on day zero. In addition, animals were right censored at the end of the follow-up period if the ERP had not exceeded 10 %. The follow-up period was seven weeks for FECRT1 (winter 2019) and 6 weeks for all the other FECRTs. So, to make the follow-up period consistent across all trials, all animals were right censored at 6 weeks.

The FECRTs for winter 2019 and summer 2020 (FECRT1 and 2) were considered to have been carried out in the same calendar year, as were the winter 2020 and summer 2021 FECRTs (FECRT3 and 4), identified as calendar year one and calendar year two, respectively. Kaplan–Meier product limit estimates were used to compare survival probabilities for ERP between treatment groups, between seasons and between calendar years (Kaplan and Meier, 1958). In this context, survival probability means the probability of remaining egg free by 6 weeks post treatment. The homogeneity of the Kaplan–Meier survival probabilities were tested using the log rank test statistic (Harrington and Fleming, 1982). The log-rank test compares the observed number of events in each group to what would be expected if the survival curves were identical. If the log rank statistic was found significant, a Cox proportional hazards model (CPH) (Fox and Weisberg, 2023) was then fitted to the same data to estimate the hazard ratio (HR). The proportional hazards assumption which is made when employing a Cox proportional hazards regression model was checked using the cox.zph function from the survival package which checks that the scaled Schoenfeld residuals are independent of time. All Cox regression models used in the analysis passed the assumption of proportional hazards.

The survival analysis was completed using the survival package (Therneau, 2023) in R (version 4.2.1; R Development Core Team (2008), R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900,051–07-0, URL http://www.R-project.org).

3. Results

3.1. Anthelmintic efficacy and egg reappearance periods

The results of the various FECRT are summarized in Tables 2 to 4, with the calculated ERP summarized in Table 5. For group 1 there was no significant difference in the starting egg counts across the 4 tests ($P = 0.38$). The slight decline in average egg count in Group 2 by FECRT4 although not significant ($P = 0.47$) was due in part to the removal of one animal from the herd for health reasons unrelated to this study, this animal having the highest count in this group in the first two tests. In addition, in FECRT4, a greater than expected number of Group 2 animals had zero counts on Day 0.

In FECRT 1 to 4, treatments with ivermectin, moxidectin or abamectin all saw counts reduce by more than 99 % in the first 4 weeks after treatment, with counts generally rising from week 5 onwards. Across all tests, a zero egg count after ML-treatment was somewhat likelier when treatments were given in winter. This manifested in the number of animals with zero counts in the first 4 weeks after treatment which were consistently 100 % for winter treatments, with more animals, although still a minority, shedding eggs at some point in those 4 weeks in summer.

In general, there was reasonable agreement between the 3 methods used to calculate ERP, with somewhat more conservative values (longer ERP) obtained using Method 1, and to an extent Method 3. The ERP for ivermectin and moxidectin varied, according to test, season and the method of calculation, from 7 to 5 weeks. Across the 4 main FECRT (1 to

Table 2
Summary of the four consecutive FECRT conducted with Group 1 horses treated with ivermectin at 200 μg/kg and sampled before and weekly for up to 7 weeks after treatment. Arithmetic (AM) and Geometric (GM) means are presented along with the per cent efficacy calculated using them, the range of egg counts seen and the number of animals with a zero count post-treatment (n 0epg), the upper 95 % confidence interval for efficacy calculated using AM and the Probability (%) of efficacy being greater than 90 %. Figures in red indicate when the data determined that the egg reappearance period had been achieved according to the different methods used.

Test	n		Pre	1	2	3	4	5	6	7
FECRT 1	12	GM	433.0	0	0	0	0	12.6	41.3	61.1
(winter)		Efficacy %		100	100	100	100	97.1	90.4	**85.8**
		AM (range)	576.6	0	0	0	0	38.2	76.5	172.8
			(34-1122)					(0-272)	(0-221)	(0-697)
		n 0epg		12	12	12	12	6	3	4
		Efficacy %		100	100	100	100	93.3	**86.7**	**70.0**
		Upper CI		100	100	100	100	95.4	**89.9**	**73.6**
		P >90%		100	100	100	100	98.3	2.4	0.0
FECRT 2	11	GM	214.1	0.9	0	1.9	0.9	31.8	120.5	
(summer)		Efficacy %		99.6	100	99.1	99.6	**85.1**	**43.7**	
		AM (range)	425.0	1.5	0	3.1	1.5	70.7	245.7	
			(17-1309)	(0-17)		(0-17)	(0-17)	(0-238)	(0-578)	
		n 0epg		10	11	9	10	4	2	
		Efficacy %		99.6	100	99.3	99.6	**83.3**	**42.2**	
		Upper CI		100	100	100	100	**87.6**	**51.8**	
		P >90%		100	100	100	100	0.1	0	
FECRT 3	11	GM	287.3	0	0	0	0	25.5	83.3	
(winter)		Efficacy %		100	100	100	100	91.1	**71.0**	
		AM (range)	485.3	0	0	0	0	52.5	210.2	
			(17-1479)					(0-170)	(0-646)	
		n 0epg		11	11	11	11	4	3	
		Efficacy %		100	100	100	100	**89.2**	**64.3**	
		Upper CI		100	100	100	100	92.2	**64.3**	
		P >90%		100	100	100	100	28.6	0	
FECRT 4	11	GM	432.0	0.9	1.3	0.9	0	64.8	267.9	
(summer)		Efficacy %		99.8	99.7	99.8	100	**85.0**	**38.0**	
		AM (range)	550.2	1.5	3.1	1.7	0	132.9	404.9	
			(153-1224)	(0-17)	(0-34)	(0-17)		(0-408)	(34-918)	
		n 0epg		10	10	10	11	3	0	
		Efficacy %		99.7	99.4	99.7	100	**75.8**	**26.4**	
		Upper CI		100	99.8	98.5	100	**80.8**	**36.8**	
		P >90%		100	100	100	100	0	0	

4) the ERP for ivermectin and moxidectin were up to 2 weeks longer in the Winter Tests (FECRT 1 and 3) in comparison to summer tests (2 and 4). The ERP for abamectin in FECRT3 was 6 weeks according to all 3 methods.

In FECRT 4, the efficacy of fenbendazole 7 days after treatment was only 52 %. Hence no ERP could be calculated. Likewise, in FECRT 5, the second abamectin-containing product was only 68 % efficacious, with only 2/12 animals having no eggs in their faeces post-treatment.

All larval cultures before and after treatment indicated only the presence of cyathostomin nematodes with no large strongyle species present.

3.2. Survival analysis results

3.2.1. Ivermectin results over FECRT 1 to 4

A Kaplan–Meier product limit estimate was fitted to the group 1 ivermectin results over the 4 FECRT (Fig. 1). The log rank statistic found evidence for a difference in the survival curves for summer and winter treatments ($p = 0.046$). The median survival time was 5 weeks for summer and 6 weeks for winter. The CPH model fitted to the same data found that there was a tendency for winter treatments to have a lower hazard for ERP (longer ERP) than summer treatments, HR = 0.5 (95 % CI 0.24–1.03, $p = 0.06$).

The log rank statistic found some evidence for a difference in the survival curves for calendar year1 and calendar year2 ivermectin treatments ($p = 0.07$). The CPH model found that there was a tendency for calendar year2 ivermectin treated animals to have a higher hazard for ERP (shorter ERP) than calendar year1, HR = 2.02 (95 % CI 0.97–4.2, p = 0.06).

The horses used in the ivermectin treatment group came from the three different grazing mobs (1–3), but there was no evidence for a difference in survival curves between the mobs ($p = 0.44$).

Table 3
Summary of the two consecutive FECRT conducted with Group 2 horses treated with moxidectin at 400 μg/kg and sampled before and weekly for up to 7 weeks after treatment. Arithmetic (AM) and Geometric (GM) means are presented along with the per cent efficacy calculated using them, the range of egg counts seen and the number of animals with a zero count post-treatment (n 0epg), the upper 95 % confidence interval for efficacy calculated using AM and the Probability (%) of efficacy being greater than 90 %. Figures in red indicate when the data determined that the egg reappearance period had been achieved according to the different methods used.

Test	n		Pre	1	2	3	4	5	6	7
FECRT 1	11	GM	262.1	0	0	0	0	1.6	15.6	28.0
(winter)		Efficacy %		100	100	100	100	99.4	93.9	**89.1**
		AM (range)	408.0	0	0	0	0	4.6	40.2	66.4
			(34-1175)					(0-51)	(0-289)	(0-340)
		n 0epg		11	11	11	11	10	4	3
		Efficacy %		100	100	100	100	98.8	90.0	**83.5**
		Upper CI		100	100	100	100	99.6	93.4	**88.3**
		P >90%		100	100	100	100	100	46.9	0.1
FECRT 2	10	GM	275.3	1.0	0	2.1	1.0	11.9	34.3	
(summer)		Efficacy %		99.6	100	99.2	99.6	95.7	**87.5**	
		AM (range)	429.7	1.7	0	3.4	1.7	78.2	124.1	
			(34-867)	(0-17)		(0-17)	(0-17)	(0-595)	(0-731)	
		n 0epg		9	10	8	9	7	4	
		Efficacy %		99.6	100	99.2	99.6	**81.8**	**71.1**	
		Upper CI		100	100	99.7	100	**86.7**	**77.5**	
		P >90%		100	100	100	100	0	0	

* For FECRT 5, post treatment samples were collected 7 (n = 9) and 15 (n = 3) days after treatment.

3.2.2. *Ivermectin versus moxidectin treatment*

A Kaplan–Meier product limit estimate was fitted to the combined data for FECRT1 and FECRT2 (Fig. 2). The log rank statistic found no evidence for a difference in the survival curves for ivermectin and moxidectin treatment (p = 0.4). However, there was some evidence to show that across the two anthelmintics there was a difference between survival curves for summer and winter treatments ($p = 0.054$), with the survival probability being much lower (shorter ERP) in summer compared to winter. The CPH model fitted to the same data found that there was a tendency for winter treatments to have a lower hazard for ERP (longer ERP) than summer treatments, HR = 0.44 (95 % CI 0.18–1.08, p = 0.07).

3.2.3. *Ivermectin versus abamectin treatment*

A Kaplan–Meier product limit estimate was fitted to the FECRT3 data (Fig. 3). The log rank statistic found no evidence for a difference in the survival curves for ivermectin and abamectin anthelmintic treatments ($p = 0.7$).

3.2.4. *Ivermectin versus fenbendazole treatment*

A Kaplan–Meier product limit estimate was fitted to the FECRT4 data (Fig. 4). The log rank statistic found evidence for a difference in the survival curves for ivermectin and fenbendazole ($p < 0.0001$). The median survival time was 1 week for fenbendazole and 6 weeks for ivermectin. The HR could not be estimated using the CPH model.

4. Discussion

As far as the authors can ascertain, this paper includes the first use of survival analysis to model equine parasites. Survival analysis is a relatively straight forward statistical method, and when you have time to event data, such as ERPs, it can be used to quickly explore differences in the data. Nevertheless, our analyses used relatively small numbers of horses, and estimates of efficacy as determined by reductions of faecal egg shedding so clearly our findings must be viewed in the light of the well-known limitations of such examinations discussed extensively elsewhere (e.g. Vidyashankar et al., 2012).

In the present study, the ERP, calculated by the currently recommended method (Method 3 - Nielsen et al., 2022c) for both ivermectin and moxidectin were between 5 and 7 weeks, findings similar to the shortened ERP for both ML reported overseas in recent years (MacDonald et al., 2023). Few studies have examined the ERP for abamectin. In one study in Australia, abamectin held egg counts to close to zero for at least 4 weeks (Holm-Martin et al., 2005). By week 6 efficacy had declined to 85 %, but the upper confidence interval was still >90 % with the latter continuing out to 12 weeks when egg count monitoring ceased. In a more recent study also in Australia, the ERP for a combination of abamectin and morantel was however found to be only 4 weeks (Abbas et al., 2021).

The finding of marked inefficacy of fenbendazole was not surprising given the historic high levels of resistance to this anthelmintic class thought to be present in this country since the 80s (Scott et al., 2015), and resistance to this drug class is clearly present in the herd even though benzimidazole anthelmintics have not been used in the horses in at least the last two decades, the long-term persistence of resistance to benzimidazoles in the absence of use of that drug class certainly having been noted by others (Lyons et al., 2007). Resistance to benzimidazoles may not however be as widespread in this country as generally believed. More recent results for oxibendazole, showed that it had efficacy of anywhere between 67 and 99 % (Morris et al., 2019).

Of the two abamectin-containing products used in this study only one was fully effective. Both were meant to deliver the same dose (200 μg/kg) of abamectin to the horses, but it would seem possible that the products were not delivering the same amount of drug to the worms themselves, one potential explanation being differences in how the products were formulated. It should be noted that the abamectin product that failed has also failed in recent studies in Australia (Abbas et al., 2021, 2024) suggesting that potential product differences will need to be considered much more critically in the future when confirming the presence of resistance.

This study generated some evidence that there can be differences between tests conducted in winter as opposed to summer, although such

Table 4
Summary of FECRT 3 to 5 conducted with Group 2 horses (FECRT 3, 4) and a mixture of horses from both groups 1 and 2 (FECRT5). Horses in FECRT 3 were treated with the first abamectin-containing product at 200 μg/kg, those in FECRT 4 with fenbendazole at 10 mg/kg, whilst those in FECRT5 were treated with a second abamectin-containing product also at 200 μg/kg. Horses were sampled before and weekly for 6 weeks after treatment. Arithmetic (AM) and Geometric (GM) means are presented along with the per cent efficacy calculated using them, the range of egg counts seen and the number of animals with a zero count post-treatment (n 0epg), the upper 95 % confidence interval for efficacy calculated using AM and the Probability (%) of efficacy being greater than 90 %. Figures in red indicate when the data determined that the egg reappearance period had been achieved according to the different methods used, or as in the case of FECRT 4 and 5 when initial efficacy was less than 90 %.

Test	n		Pre	1	2	3	4	5	6
FECRT 3	11	GM	159.0	0	0	0	0	7.7	30.9
(abamectin –		Efficacy %		100	100	100	100	95.1	**80.5**
Product 1)									
(winter)		AM (range)	360.1	0	0	0	0	30.9	92.7
			(17-1904)					(0-238)	(0-459)
		n 0epg		11	11	11	11	8	4
		Efficacy %		100	100	100	100	91.4	**74.2**
		Upper CI		100	100	100	100	94.4	**80.4**
		P >90%		100	100	100	100	69.9	0
FECRT 4	7	GM	117.4	61.5	98.9	118.2	60.5	82.4	129.3
(fenbendazole)		Efficacy %		47.6	15.8	0	48.4	29.8	0
(summer)		AM (range)	250.1	119	189.4	206.8	204.0	182.1	269.6
			(17-629)	(0-204)	(0-357)	(17-425)	(0-544)	(0-493)	(0-901)
		n 0epg		2	1	0	2	2	1
		Efficacy %		**52.4**	**24.3**	**17.3**	**18.4**	**27.2**	**0**
		Upper CI		**65.5**	**42.7**	**46.8**	**38.4**	**45.0**	**21.9**
		P >90%		0	0	0	0	0	0
			Pre	Post*					
FECRT 5	12	GM	523.7	93.3					
(abamectin -		Efficacy %		82.2					
Product 2)		AM (range)	640.3	206.8					
			(51-1122)	(0-646)					
		n 0epg		2					
		Efficacy %		**67.7**					
		Upper CI		**73**					
		P >90%		0					

* For FECRT 5, post treatment samples were collected 7 (n = 9) and 15 (n = 3) days after treatment.

Table 5
Summary of the reported Egg reappearance periods (in weeks) after treatment with ivermectin (200 μg/kg), moxidectin (400 μg/kg), and abamectin (200 μg/kg – first product) in consecutive Faecal egg count reduction tests. ERP was calculated by three methods based on when efficacy calculated using the geometric mean (Method 1) or arithmetic mean (Method 2) counts or the upper 95 % confidence interval for efficacy fell to less than 90 % (Method 3).

	Ivermectin				Moxidectin		Abamectin
FECRT	1	2	3	4	1	2	3
Method 1	7	5	6	5	7	6	6
Method 2	6	5	5	5	7	5	6
Method 3	6	5	6	5	7	5	6

differences were subtle and shown to be of only marginal significance statistically (P slightly below or above 0.05). With egg counts essentially zero for the same amount of time after effective ML treatment (at least 4 weeks), any differences in ERP in our study depended principally on how quickly counts rose in week 5 and onwards, and counts tended to rise more quickly in summer tests, most noticeably so for moxidectin which had a 7 week ERP in winter, 5 weeks in summer. We note that this study is likely not the first to have noted a seasonal difference for ERP, in the study conducted by Sokol et al. (2015), there was likely a difference in the ERP of spring and autumn ivermectin treatment had they examined this specifically.

Shortened ERP were originally ascribed to reduced efficacy against cyathostomin L4 stages resident in the lumen of the gut at the time of treatment, although this view has been challenged recently (Nielsen et al., 2022a). To our minds, the simplest explanation of the present findings would be variation in the number of late L4 surviving treatment, since any that do survive will logically be the first to lay eggs and if more are surviving, egg counts would be expected to rise more quickly. It must be acknowledged that the exact extent of any inefficacy against L4 in our study remains unknown, but reduction in the efficacy of ML against late L4 has certainly been demonstrated by others (Nielsen, 2022).

If the rate of rise of egg counts after effective adulticide treatment is indeed dependent on the number of later L4 surviving treatment, then this likely depends on two things: the number of those stages present when treatment is given, and then, the efficacy of treatment itself. This raises the question of whether the differences observed in the present study were due to variance in parasite number or the efficacy against them, although undoubtedly given the limitations inherent in the

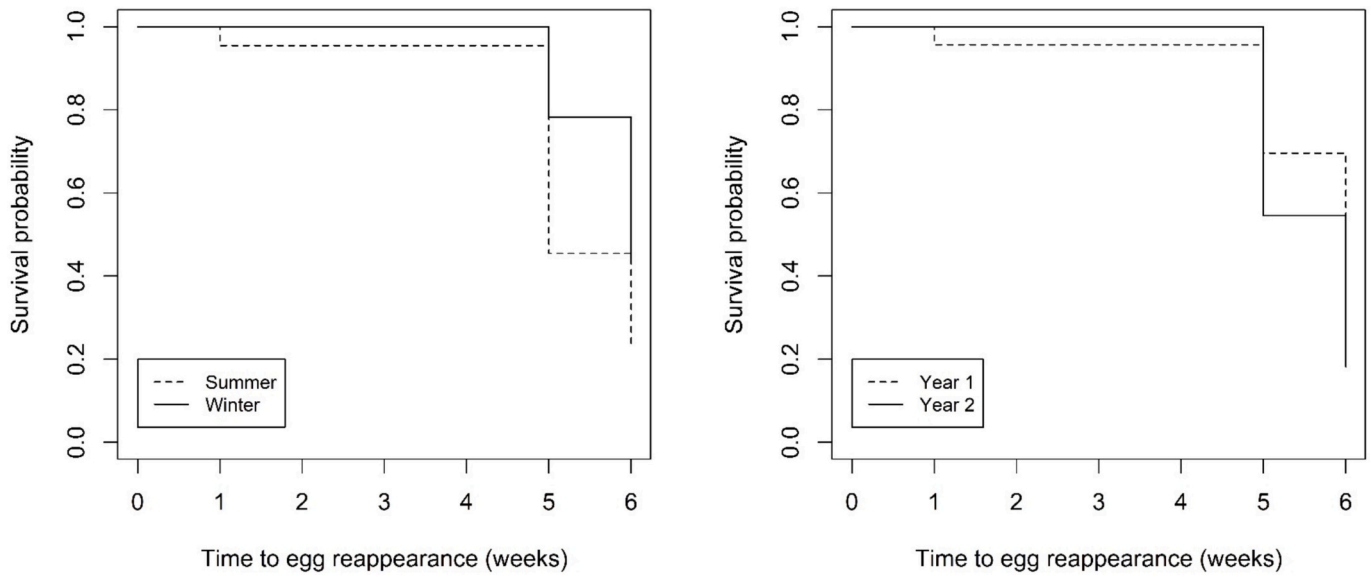

Fig. 1. Kaplan–Meier survival curves for ivermectin comparing effect of season (left) and year (right) on egg reappearance times for a group of horses treated with ivermectin, 4 times, in winter then summer for two years, with egg counts monitored before and weekly after treatment.

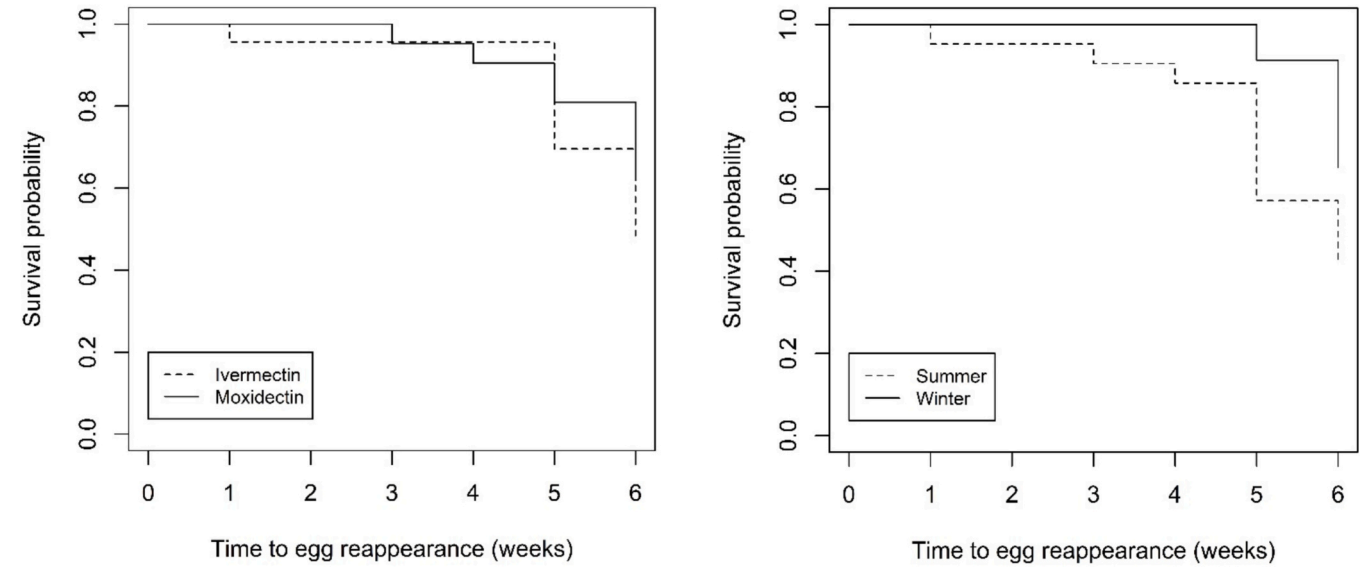

Fig. 2. Kaplan–Meier survival curves comparing ivermectin and moxidectin (left) and effect of season (right) on egg reappearance times for two groups of horses treated respectively with ivermectin or moxidectin, 2 times, in winter then summer, with egg counts monitored before and weekly after treatment.

methodology employed, this question would not be answered in this study.

The possibility that the differences we observed in ERP were instead due to variation in the prevalence of different cyathostomin species or strains between seasons, between those with shorter and longer development times, cannot be ruled out, but we wonder if this should rather have translated to earlier detection of eggs in summer tests, rather than the same relative period of egg absence. Other factors such as variation in the fecundity of female worms may also have played a role.

An additional difference that trended towards significance in the present study was the observation that egg counts appeared to be rising more quickly in the second year of testing, something that would be theoretically at least consistent with a further decline of efficacy against L4 over time.

Regardless of how many L4 survive treatment, they still appear to take at least 4 weeks to finish their maturation and commence egg laying, and this suggests that ERP should be unlikely to decline much further. An obvious next step in resistance development will of course be the occurrence of resistance in the egg-laying adult stages, but this obviously will manifest not as a further shortening of ERP, but as a failure to adequately reduce egg counts in the first place. Whilst ERP as short as 4 weeks have been reported overseas (MacDonald et al., 2023), these could be associated with different cyathostomin species or indeed strains with shorter development times.

5. Conclusion

Following treatments with ivermectin, moxidectin and at least one abamectin product, egg counts were near zero for at least 4 weeks and with ERP of 5 to 7 weeks. Egg counts tended to rise more quickly when treatments were applied in summer, and in the second year of testing as opposed to the first. Treatment with fenbendazole and a second

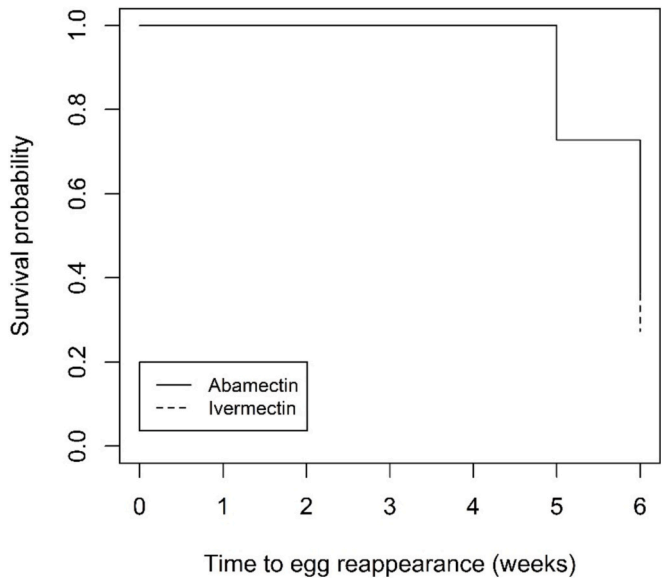

Fig. 3. Kaplan–Meier survival curves comparing ivermectin and abamectin treatment of two groups of horses in winter and the effect on egg reappearance times, with egg counts monitored before and weekly after treatment.

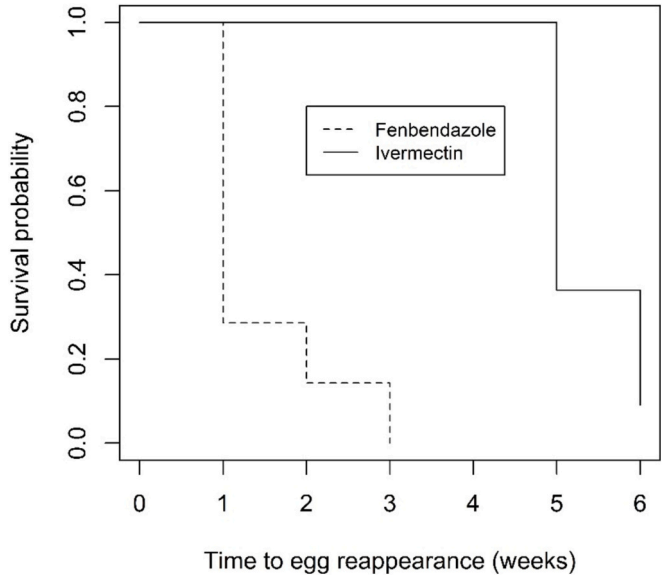

Fig. 4. Kaplan–Meier survival curves comparing ivermectin and fenbendazole treatment of two groups of horses in summer and the effect on egg reappearance times, with egg counts monitored before and weekly after treatment.

abamectin product were ineffective.

Ethics statement

All procedures were approved by the Massey University Animal Ethics Committee, protocol 19/58.

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Data statement

Full data from this study can be made available on reasonable request.

CRediT authorship contribution statement

Ian Scott: Writing – original draft, Investigation, Data curation, Conceptualization. **Kevin E. Lawrence:** Writing – review & editing, Formal analysis. **Erica K. Gee:** Writing – review & editing, Investigation.

Declaration of competing interest

The authors declare no conflict of interest.

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