Anthelmintic resistance in equine nematodes: do shortened egg reappearance periods show seasonal variation

Final Report

Ian Scott

Tāwharau Ora - School of Veterinary Science, Massey University, Palmerston North

Lay summary

The small strongyles, also known as the cyathostomes, are now the major parasites of horses, representing close to 100% of the roundworm parasites present in animals kept at pasture in most countries including Aotearoa, New Zealand. In recent years, these parasites have shown signs that they are now less susceptible to the effects of the drugs, anthelmintics, used to remove them from the guts of horses than they used to be. Of particular concern is the decline in efficacy of a group of anthelmintics, the macrocyclic lactones (ML), that for a long time have been the major type of anthelmintic used in horses. The fear being that when these anthelmintics fail, there are limited alternatives for parasite control in this species. In most cases, the ML anthelmintics have remained able to remove the adult, egg laying parasites that horses harbour, but what does appear to have changed is that these chemicals now have a diminished effect on the earlier, juvenile, or larval stages of the lifecycle. Following treatment of the horse, larval stages that were not removed eventually mature and start laying eggs and the diminished susceptibility of larvae has resulted in a shortening in the amount of time required for egg counts to begin to rise again after treatment – shortening of the so called egg reappearance period (ERP).

It is known that the worm population of horses can change between seasons, with more larval stages present in winter than in summer. The difference has generally been attributed to an accumulation in winter of larvae that are not developing, so called inhibited larvae, and it has been assumed that otherwise, larvae that are not inhibited take the same amount of time to complete their development at all times of the year. So far however no studies have examined the potential for seasonal differences in ERP. To this end, a series of 4 tests were conducted to examine the efficacy and ERP of 3 different ML anthelmintics in two groups of horses. Comparison was made

between ivermectin and moxidectin, the latter historically associated with a much longer ERP due in large part to its greater persistence in the tissues of treated animals, and between ivermectin and abamectin, and also with the anthelmintic fenbendazole which is in a different anthelmintic class to the other 3. Ivermectin was itself tested over all 4 tests, which were conducted in consecutive winters and summers over two years. The tests monitored the numbers of parasite eggs present in faecal samples of the horses before and then weekly after treatment. Efficacy against the egg laying adults was judged by how much counts declined immediately (one to two weeks) after treatment and ERP was judged by how long it took for egg counts to rise after treatment to 10% or more of what they had been before treatment.

In addition to the above, in further tests, the efficacy of a second abamectin-containing product was examined and that of a product containing a combination of pyrantel and oxibendazole.

Treatment with ivermectin, abamectin and moxidectin were all capable of reducing egg counts by close to 100% for at least 4 weeks after treatment, thereafter, counts began to rise with ERP for the 3 ML anthelmintics of approximately 5-7 weeks over the 4 tests, with no significant differences evident between the different products. No ERP has been published for abamectin, but the observed ERP for ivermectin and moxidectin were shorter than those reported for these products when they were first available, particularly so in the case of moxidectin.

Egg counts did not disappear after treatment with fenbendazole so no ERP was calculable for this drug. This was also observed with the second abamectin product tested which also proved ineffective. The difference between the two abamectin-containing products was most likely due to one of the products not being able to get the same concentration of drug to the worms despite the dose rates being the same. This could be most readily explained by differences in solubility of the two products due to differences in formulation.

The pyrantel/oxibendazole combination reduced eggs almost as well as the ML anthelmintics, but counts started to rise earlier in the 4th week. Nevertheless the ERP observed was similar to those of the ML.

Over the course of the 4 main tests, there was the suggestion that ERP were shorter in summer than in winter and that ERP were shorter in the second year of testing. The reason for a shorter ERP in summer was a faster rise in egg counts, rather than egg counts starting to rise earlier, and the likeliest explanation for this being that in summer there would be more larvae present and hence left behind after treatment than in winter. The further shortening in ERP across the 4 tests could however be seen as evidence of ongoing declines in efficacy against the larval stages.

Shortened ERP are often seen as an early warning of the development of parasite resistance to anthelmintics, yet, arguably, since larval stages usually outnumber the adults in most horses, shortened ERP likely indicate that resistance is already quite advanced. At some point even the adults will be harder to kill, something further heralded by the failure of the second abamectin-containing product.

Abstract

In this study we investigated the egg reappearance periods (ERP), often seen as an early warning of anthelmintic resistance development, of a variety of commonly used equine anthelmintics. We also investigated whether ERP might show seasonal variation. In the main part of the study, four Faecal egg count reduction tests (FECRT1-4) were conducted in Winter (Jun/Jul) 2019, Summer (Jan/Feb) 2020, Winter 2020 and Summer 2021 respectively. 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 – a common definition of ERP. In addition to the above, in Spring 2021, we tested the efficacy of a second abamectin-containing paste formulation, and we also completed an additional FECRT in Autumn 2021 to assess the efficacy and ERP for a pyrantel embonate/oxibendazole combination. Treatment with ivermectin, moxidectin and the first abamectin product all reduced egg counts by >99% for 4 weeks after treatment, and there were no significant differences between the 3 treatments, with ERP, judged as the first week when counts exceeded 10% of pre-treatment, of 5-7 weeks. There was a tendency for counts to rise more rapidly in summer and counts also tended to rise more quickly in the Winter 2020 and Summer 2021 tests as opposed to the first two tests. Both the second abamectin product and the fenbendazole were in contrast ineffective, reducing egg counts immediately after treatment by 68 and 52% respectively. The pyrantel/oxibendazole combination had slightly lower initial efficacy, with counts starting to rise in the 4th week after treatment, although the ERP was similar to those reported for the ML anthelmintics. The shortened ERP recorded in this study are consistent with reduced anthelmintic efficacy against later larval stages. The faster climb of egg counts in summer tests suggest that more of the later larval stages are present in summer than in winter, whilst the higher rates of increase in the last two tests suggest a slight decline in efficacy over the course of the 2 years of the study. Shortened ERP and the failure of the second abamectin product both suggest that resistance to the ML class is increasing.

Introduction

Anthelmintic efficacy in equines is most frequently judged by examining how much egg counts decline after treatment. Efficacy is thus 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 when egg counts are reduced to zero or near-zero after treatment, the amount of time required for egg counts to return, the egg reappearance period (ERP), is often now shortened. Shortened ERP have arisen when efficacy has declined against the earlier larval stages, whilst still remaining good against the egg laying adults. Worldwide, many studies have shown the existence of shortened ERP, but few studies have looked at how repeatable ERP are, and a further unanswered question is whether there is a seasonal influence on ERP. Horses are fairly unique amongst domestic animal species in that adult parasites typically make up less than 10% of the total worm burden present, and it is known that this percentage declines in winter when more encysted larval stages are present. It is known that part of the reason for the accumulation of larval stages over winter is that many early L3 stages become inhibited in development at this time, but it is also possible that the later larval stages may be slower to develop at this time. If so, then this could see ERP prolonged in winter.

The study reported here was designed to examine the efficacy and ERP of three commonly used anthelmintics of horses in the Macrocyclic lactone class – ivermectin, moxidectin and abamectin - and one anthelmintic in the benzimidazole class – fenbendazole. The products were tested in four FECRT conducted in winter and summer over the course of 2 years. In addition to these tests, the opportunity was taken to also examine the efficacies (and ERP if relevant) of a second, registered abamectin containing product for horses and a combination product containing pyrantel embonate and oxibendazole.

Methods

The same group of horses, with minor additions and losses over time, were used in the tests and were divided into two groups based on initial egg counts. The average age of the horses at the commencement of FECRT1 was 14.9 years (range 8 to 22). One Group 2 horse was an ex-Kaimanawa horse whose exact age is unknown, but it has been with the herd for a number of years. The horses weighed on average 540, 547, 522 and 562 kg at the start of FECRT 1 to 4 respectively.

In all 4 tests the same horses (Group 1) received ivermectin (Equimax LV®, Virbac), whilst the horses in Group 2 received moxidectin (Equest Plus Tape®, Zoetis - FECRT 1,2) and then abamectin (Genesis Horse Wormer® - FECRT3) and later fenbendazole (Panacur 100®, MSD Animal Health - FECRT4). Across the 4 tests, 1, 3, 1 and 4 horses were excluded from analyses due to having zero counts on the day of treatment. The test for the efficacy of a second abamectin containing product (Farnam MecWorma and Bot broad Spectrum Worm Paste for Horses®, International Animal Health Products) was conducted approximately 2 weeks after the completion of FECRT3. 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. The final test, for the pyrantel embonate/oxibendazole combination (Strategy T), was conducted one week after FECRT4 was completed. Both additional tests used a mixture of the 12 animals with higher counts from both groups 1 and 2. The rationale for the final test was to examine the efficacy and ERP of pyrantel, but since no preparation of pyrantel on its own is available in this country, the combination product was chosen.

All products (bar fenbendazole) used were registered paste formulations delivered orally by syringe and the ivermectin, moxidectin and abamectin-containing products also included praziquantel. Praziquantel is included for efficacy against cestode parasites (tapeworms) and has no efficacy against nematode parasites. Fenbendazole was administered in feed. One animal refused to eat the feed to which the fenbendazole had been added and was dosed with ivermectin instead. In treating the horses, all received a dose volume rounded up to the next 50kg representing the next notch on the syringe, i.e. 501 and 540kg horses would both be given doses sufficient for a 550kg bodyweight.

The (minimum) dose rates for each anthelmintic were thus:

Ivermectin	200µg/kg			
Moxidectin	400µg/kg			
Abamectin	200µg/kg			
Fenbendazole	7.5mg/kg			
Pyrantel embonate	13mg/kg	+	Oxfendazole	10mg/kg

Faeces were collected from the rectum of each animal or picked up off a clean concrete floor immediately after deposition. Egg counts were done weekly using 2g of faeces from each animal with a 3-chambered McMaster slide such that each egg counted represented a count of 17 eggs per gram. Larval cultures were prepared using faeces not used for egg counts.

Efficacy was calculated each week after treatment as the percent of eggs removed by treatment according to the following formula:

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

The egg reappearance period was calculated by three methods. The first method (Method 1) simply recorded the first week when egg counts had risen to 10% or more of the pre-treatment values, using both Arithmetic (AM) and geometric mean (GM) counts. This is equivalent to calculating when efficacy had declined to less than 90%. The second method (Method 2) used regression lines of arithmetic mean counts, to attempt to calculate a more precise figure for when egg counts rose to actually meet 10% and 20% of the pre-treatment counts.

A third method (Method 3) ignored pre-treatment egg counts and simply calculated regression lines for post-treatment egg counts to find a prediction of the time required for average (AM) counts to reach 200epg (a figure commonly used as a cut-off to decide whether anthelmintic treatment is necessary or not).

Statistical analysis of the Egg reappearance periods utilised Kaplan-Meier product limit estimates to compare "survival" probabilities between treatments, season (summer, winter) and year (with FECRT 1 and 2, and 3 and 4 considered to have been conducted in two consecutive years). A Cox Proportional Hazards (CPH) model was then fitted to the data.

Results

FECRT 1 to 4

The starting (Day 0) egg counts (epg) in all four of the main FECRT (1-4) were as follows:

		FECRT1 Winter 2019	FECRT2 Summer 2020	FECRT3 Winter 2020	FECRT4 Summer 2021
Group 1	AM	576 (n=12)	425 (n=11)	485 (n=11)	550 (n=11)
	Range	34-1119	17-1309	17-1479	153-1224
	GM	430	214	287	432
Group 2	AM	402 (n=11)	430 (n=10)	360 (n=11)	250 (n=7)
	Range	34-1352	34-867	17-1904	17-629
	GM	258	197	159	117

At least for group 1 there was no significant difference in the starting egg counts across the 4 tests. The decline in average egg count in Group 2 in FECRT 3 and 4 was at least in part due to the removal of one animal from the herd for reasons unrelated to this study. This animal had the highest count in this group in the first two tests. In addition, in FECRT4 a number of Group 2 animals also had zero counts on Day0 and one animal had refused to consume the fenbendazole-laced feed.

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. In contrast the efficacy of fenbendazole 7 days after treatment was only 52%. Hence no ERP could be calculated for fenbendazole.

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

The results for ERP for FECRT1 to 4 calculated as first week in which average egg counts were 10% or more of those pre-treatment (Method 1) are presented as follows.

	FECRT1 Winter 2019	FECRT2 Summer 2020	FECRT3 Winter 2020	FECRT4 Summer 2021
Ivermectin (Gp1)				
AM	6	5	5	5
GM	7	5	6	5
Moxidectin (Gp2)				
AM	7	5		
GM	7	6		
Abamectin (Gp2)				
AM			6	
GM			6	

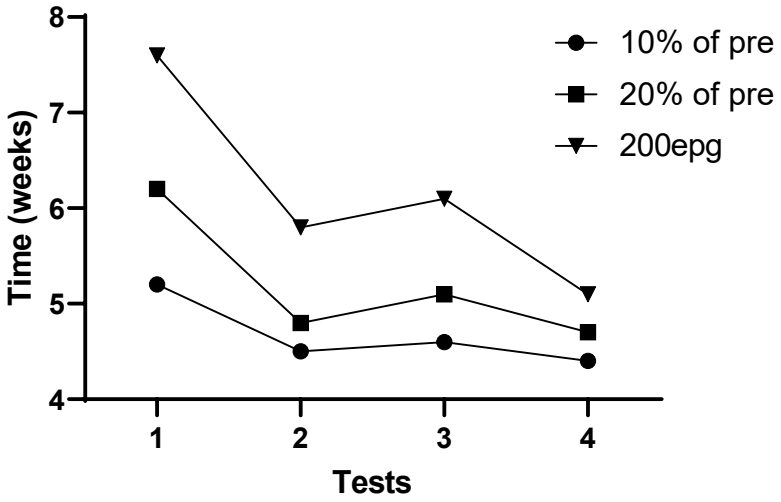
The following shows the ERP calculated using regression lines for time for egg counts to reach 10% of those pre-treatment (Method 2):

	FECRT1 Winter 2019	FECRT2 Summer 2020	FECRT3 Winter 2020	FECRT4 Summer 2021
Ivermectin (Gp1)	5.2	4.5	4.6	4.4
Moxidectin (Gp2)	6.1	4.6		
Abamectin (Gp2)			4.9	

The following shows the ERP calculated using regression lines as time for average counts to reach 200epg (method 3):

	FECRT1 Winter 2019	FECRT2 Summer 2020	FECRT3 Winter 2020	FECRT4 Summer 2021
Ivermectin (Gp1)	7.6	5.8	6.1	5.1
Moxidectin (Gp2)	13.0	7.5		
Abamectin (Gp2)			8.4	

The following figure shows a comparison of the time calculated from regression lines (Method 2) for arithmetic mean egg count to exceed 10% and 20% of the pre-treatment counts and for egg counts to rise to 200epg for treatment with ivermectin across FECRT 1 to 4.



Comparing the results of FECRT 1 and 2, the CPH model showed no significant difference between ivermectin and moxidectin treatment over the 2 seasons, p=0.38. However, there was a tendency for winter treatments to have a lower hazard for ERP (longer ERP), hazard ratio = 0.44 (95% CI 0.18 – 1.1), p=0.075.

Applied over the 4 FECRT (2 years, 4 seasons) to only the ivermectin treatment data, the results showed a tendency for year 2 (winter 2020 and summer 2021) to have shorter ERP, hazard ratio = 2.05 (95% CI 0.98 – 4.3), p=0.06, and a tendency for winter treatments to longer ERP, hazard ratio = 0.49 (95% CI 0.24 – 1.02), p=0.06.

In FECRT 3 there was no significant difference between ivermectin and abamectin treatment, p=0.72.

Additional tests

Efficacy of the second product was only 68%, with only 2/12 animals having no eggs in their faeces post-treatment.

Treatment with the pyrantel embonate/fenbendazole combination was associated with lower initial efficacy in the first 3 weeks post-treatment (>98, <100%), before counts started to rise in the fourth

week. By week 5 (week 6 using GM) the 10% threshold had been reached and the ERP (10%) calculated using the regression line was 4.7 weeks.

Discussion and conclusions

ML anthelmintics remain highly effective against adult cyathostomin parasites, but ERP are reduced and there is now little (no) difference in ERP between ivermectin, moxidectin and abamectin

All ML treatments remained highly effective against cyathostomin adults, removing >99% of the eggs in all tests (bar the test for the second abamectin product). This finding is in keeping with overseas studies in which shortened ERP have also been reported. Indeed the suggestion is that in any animal(s) for which lower initial efficacy is observed, the cause is often more likely to be inadequate dosing (weight underestimated or part of dose spat out) than overt resistance.

Thus far there have been no confirmed reports of overt ML-resistance in cyathostomins in New Zealand although there have been reports overseas (Nielsen et al., 2020). Morris and colleagues (2019) found no evidence of ML-resistance in strongyle (presumed to be cyathostomin) egg count reductions in their 2011 study conducted in 3 separate regions of the country. In another study, carried out across six Waikato stud farms, the efficacy of ivermectin against cyathostomins was determined to be 100% for the vast majority of treated young 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 (Rosanowski et al., 2017). More worrying findings were however reported recently by Blue (2017), in which the efficacy of both abamectin and moxidectin in horses in the Franklin district were lower, but still generally >90%. These latter findings however would require further investigation before resistance could be confirmed.

In the present study the ERP for ivermectin and moxidectin were shorter than the original ERP reported when the drugs were first introduced. These initial ERP have been reported as 9-13 weeks for ivermectin and 16-22 weeks for moxidectin[1]. The present study is thus far the only one that has examined an ERP for abamectin, so no comparison is possible.

[1] **Anonymous**. AAEP Parasite Control Guidelines. http://www.aaep.org/custdocs/ParasiteControlGuidelinesFinal.pdf (accessed 19 April 2021) American Association of Equine Practitioners, Lexington, KY, USA, 2019

In the early 2000s the LATU horses were treated with moxidectin and post treatment egg counts were zero / near zero for more than 20 weeks (unpublished data). Whilst the history of the herd since that time, with a reasonably regular turnover of horses, could be consistent with the importation of animals carrying more resistant worms, there are other aspects of this herd's history that would support the conjecture that shortened ERP have been hastened by practices carried out on that property over the years. At one point in time the horses were being given oral doses of an ovine, long-acting injectable preparation of moxidectin that was later shown to be only 70% effective at reducing egg counts, whilst a registered oral ML preparation was fully effective. It is likely that, due to inappropriate use of the sheep product, such practice was associated with the worms being exposed to lower concentrations of drug, a factor that has been strongly associated with the development of resistance in other species.

There may be seasonal differences in ERP, but a definite lack of season on egg counts

The standard method of calculating ERP - taking the first week post-treatment for egg counts to reach or exceed 10% of what they had been before treatment - is perhaps a crude estimate of ERP. Considering these estimates of ERP, despite an apparent reduction of ERP for ivermectin and moxidectin from FECRT1 to 2 of 1 and 2 weeks respectively, there did not appear to be any further significant changes over the subsequent tests. In contrast, the use of regression lines to calculate ERP suggests that there may in fact have been some impact of season and this was arguably more evident when the time to exceed 20% of the pre-treatment levels or when post-treatment counts would reach an average of 200epg were considered (see earlier figure). These data suggested that ERP tended to be shorter in summer than in winter. This was supported by the results of the Cox Proportional Hazards model, although the differences achieved only borderline significance.

Since egg counts were essentially zero for the same amount of time (at least 4 weeks)[2] in all tests involving ivermectin, abamectin or moxidectin, any differences in ERP depended principally on how quickly counts rose in week 5 and onwards. Differences in ERP were thus likely not due to more rapid development of larval stages, but rather to the number of these stages left behind by treatment, or at least how many eggs they ultimately produced.

Shortened ERP is thought to result from reductions in efficacy against cyathostomin later L4 stages resident in the lumen of the gut at the time treatment is given, but the exact efficacies of the

[2] From the regression lines, the x-intercepts, i.e. the time from which counts are predicted to have started to rise, for ivermectin in FECRT1 to 4 respectively were 4.2, 4.1, 4.2 and 4.1 weeks. For moxidectin in FECRT1 and 2 the figures were 4,3 and 3.9 weeks, and for abamectin (FECRT3) 4.1 weeks.

treatments in this study against these stages are unknown. The likeliest explanation of the differences observed in the study is that in summer there are more L4 present, possibly reflecting higher pasture contamination, than in winter. The data also suggests, that over the course of the tests over 2 years, there may also have been a gradual worsening of inefficacy against L4. This is suggested by comparison of the ERP winter to winter or summer to summer, both of which appear to have shortened with time.

In recent experiments carried out overseas to ascertain the efficacy of moxidectin against later L4 stages, as an explanation for shortened ERP, Bellaw et al. (2018) and Reinemeyer et al. (2015) observed that efficacy had declined by only small amounts, from what should have been close to 100% efficacy to 98.3 and 97.6% respectively. Thus even small numbers of surviving later L4 can be enough for egg shedding to be starting earlier.

Egg counts might rise quicker post-treatment in summer as opposed to winter, but the Day 0 egg counts of the Group 1 horses showed no variation between the seasons. It is generally assumed that as for ruminant nematode parasites, the number of infective equine parasite larvae on pasture will be at their lowest in winter, yet the fact that egg counts in winter were not lower reflects the fact that after egg laying adult parasites are removed by treatment, the next generation of adults comes from larval stages left behind after treatment, rather than being from larvae ingested off pasture.

ERP may not shorten any further

The present study suggests that however many L4 survive treatment with drugs like ivermectin, they still take at least 4 weeks to finish their maturation and commence egg laying, and this further suggests that ERP will 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 shortened ERP, but as a failure to adequately reduce egg counts in the first place.

BZ resistance is still present in the herd

The finding of marked resistance to fenbendazole was not surprising given the historic high levels of resistance to this anthelmintic class thought to be present in New Zealand since the 80s, and resistance to this drug class is clearly present in the LATU herd even though benzimidazole anthelmintics have not been used in the horses in the last two decades at least.

Resistance to benzimidazoles may not however be as widespread in New Zealand as generally believed. Recent results from the Franklin district of the North Island showed that fenbendazole was effective in reducing egg counts in 19 horses by on average 89% (Blue 2017), although oxfendazole appeared somewhat less effective (77%). Similar findings were recorded separately for oxibendazole, which had efficacy of anywhere between 67 and 99% (Morris et al., 2019).

Different anthelmintic formulations may not be equally effective

Two different abamectin-containing products were used in this study and only one was fully effective. Both were meant to deliver the same dose (200µg/kg) of abamectin to the horses, but it would seem clear that the products were not delivering the same amount of drug to the worms themselves (assuming no actual differences in the abamectin molecules). Experiments in ruminants have shown that different routes of administration (oral, injectable or pour-on) deliver different amounts of drug to the worms affecting efficacy often markedly, yet obviously both abamectin products in this study were oral treatments. Nevertheless, the likeliest difference for the present findings is indeed that the two products did not allow abamectin to reach the same concentration inside the worms themselves.

It must be assumed that the second abamectin product was fully effective in the past, but this may have been against parasites that were not as advanced on their path to overt resistance. Use of such a product which may be effectively underdosing animals could help select for resistance in the parasites. Underdosing is a factor in accelerating resistance precisely because it may still allow high efficacy against susceptible worms but offers poorer efficacy against resistant phenotypes, especially worms that are partially resistant. Failure of the second product to reduce egg counts is also perhaps clear evidence that we are closer to the day that other products as yet still fully effective will eventually fail.

Pyrantel is still effective (at least at LATU)

It appears that the pyrantel/oxibendazole combination was adequately effective in reducing egg counts immediately post-treatment, but suppressed egg output for 3 weeks rather than 4, with the ERP calculated to be in the order of 4-5 weeks. This figure would be compatible with the expected

ERP for either drug when working alone[3], but given the undoubted presence of BZ resistance in this herd the efficacy of the oxibendazole/pyrantel combination is likely due mostly to the pyrantel, although there is some evidence that oxibendazole is more efficacious than fenbendazole against worms that are partially resistant to that anthelmintic class.

There are scant reports of the current levels of efficacy of pyrantel in New Zealand, however, Morris and colleagues (2019) reported only 75% efficacy presumably against cyathostomins for a pyrantel/oxibendazole preparation, although this involved data from only 4 horses. With BZ-resistance potentially still widespread, and with ML-resistance developing, pyrantel and the related morantel remain the only other licensed drugs available for use in horses.

Shortened ERP question the larvicidal claims for some products

The ML anthelmintics remain highly effective against egg-laying, adult cyathostomin parasites, but reduced ERP highlight their loss of efficacy against larval stages. Whilst ivermectin and abamectin never had efficacy against encysted cyathostomins, moxidectin had some, but if the efficacy of moxidectin has now declined against emerged L4 stages, it seems reasonable to question how effective moxidectin currently is against the encysted stages, especially the most hard to kill stage the inhibited L3. Indeed, in a recent study in the USA, moxidectin had little efficacy against inhibited L3 when efficacy was judged by comparison of arithmetic mean counts (Reinemeyer et al., 2015). If this is similarly shared with other cyathostomin populations showing reduced ERP, then the worry is that currently there are no means of removing these stages from animals. This has consequences not only for the treatment and prevention of larval cyathostominosis, but also in quarantine treatment of horses transported between properties. Can any such treatment be expected to work?

To be fair, the efficacy of moxidectin against inhibited L3 has perhaps long been overestimated. Reliance on comparing GM counts to calculate efficacy can tend to overestimate efficacy and underestimate resistance problems. In one study in which GM worm burdens were compared between moxidectin-treated and untreated horses, efficacy was calculated as >90% (Bairden et al., 2006). However when comparing AM burdens, efficacy was only 54%, and a 95% confidence interval calculated for efficacy using the published worm counts included zero efficacy.

---

[3] **Anonymous**. AAEP Parasite Control Guidelines. http://www.aaep.org/custdocs/ParasiteControlGuidelinesFinal.pdf (accessed 19 April 2021) American Association of Equine Practitioners, Lexington, KY, USA, 2019

Just what level of efficacy is actually achievable currently?

It does perhaps need to be emphasised that the demonstration of efficacy against adults is misleading since it is known that the adult parasites typically only represent a minority of the population harboured by horses - in many studies the adults being less than 10% of the total burden. For any treated animal egg counts may well decline to zero after treatment, but the vast majority of the animal's burden could still be intact.

Historically, pyrantel had lower efficacy against parasite stages other than the egg-laying adults, even in the absence of resistance, and it appears that nowadays with shortened ERP the macrocyclic lactone (ML) anthelmintics like ivermectin and moxidectin, offer little advantage in respect of efficacy against L4. As also found, the inefficacy of a different abamectin formulation even against adult cyathostomins suggests however that the good efficacy of ML against adults should no longer be taken for granted. It is surely only a matter of time before resistance in the adult parasites manifests in this country as well.

Ultimately, rather than being an early warning of resistance, shortened ERP actually indicate that significant inefficacy is already present.

Does any of this matter?

Larval cultures performed throughout the study failed to detect any larvae of the much more pathogenic nematode of horses *Strongylus vulgaris*. Nor, with most horses being treated at least twice a year with praziquantel, were any eggs of the tapeworm *Anoplocephala perfoliata* detected. As with *S.vulgaris*, infections with the tapeworm of horses are recognised as being capable of serious disease even death. In our study, all larvae were identified as belonging to the cyathostomin group, which are recognised as pathogenic principally in the syndrome of larval cyathostominosis, in which large numbers of previously inhibited larvae resume development *en masse*. This syndrome is however recognised as being a greater threat to young horses, none of which are present in the LATU herd. In the study reported here, the anthelmintics given to the horses as part of the FECRT were the only ones administered across the two years of the study. There were no concerns raised about the welfare or clinical wellbeing of the horses (at least none that might be attributable to parasites), and all horses maintained reasonably even bodyweights and body conditions, although

there was a tendency for some to be slightly lighter in winter and fatter in summer, but this was largely attributed to feed availability.

From observations of the herd in this study and at other times, it can take two to three months for a horse's egg counts to return to what they had been prior to treatment, but then they often persist relatively unchanged until the next treatment is given. As in other studies, we also observed some horses to maintain similar low counts at all times whilst others consistently yielded much higher numbers of eggs. There was no indication that horses that maintained the higher counts were at a disadvantage clinically. In a recent study overseas, the performance of Standardbred trotting horses in racing was not affected by higher faecal nematode egg shedding (Fog et al., 2011) and in fact, those placing in the top three in races tended to have higher egg counts. In a similar vein, Silva and colleagues (2016) attempted to assess the digestive efficiency of horses before and after anthelmintic treatment and could find no impact of treatment on this and a variety of other health parameters, despite the horses initial egg counts exceeding 6000 eggs per gram, approximately 4 times as many as the highest numbers recorded in the LATU animals.

As discussed earlier, egg counts give no indication of the numbers of larval stages present in a horse and thus egg counts predict little of an animal's parasite status. There is one thing that egg counts do provide accurate information on however and that is the contribution to pasture contamination that any one horse makes. Allowing animals to release large numbers of eggs onto pasture for extended periods as almost certainly occurred in this study, may have no adverse consequences for more mature horses, at least not in the short term, but importantly could be of greater threat to any young horses grazed alongside them. To have large numbers of inhibited larvae resume development then of course large numbers of infective larvae must first have been accumulated off pasture.

Giving fewer than 2 treatments a year has been recognised as potentially allowing *Strongylus vulgaris* to reappear as an important parasite of horses. Nevertheless, some horses, by no means to say all, may well be able to be treated only twice a year, perhaps even less, rather than the usual 6 or more treatments per year, and still maintain good health. Such lower anthelmintic use should be expected to reduce the risk of further declines in anthelmintic efficacy.

Future work:

There is considerable scope for further work in equine parasitology, especially since at this point in time it seems unlikely that any new anthelmintics will become available for use in horses should

resistance to existing products become widespread. Some of the main ones to come directly from the work we conducted include:

- What is the efficacy of the various ML drugs against the larval cyathostome stages? Such work would involve worm counts performed on animals killed after treatment and comparison made to the counts in untreated controls. In particular, what is the efficacy of such treatments against later L4 stages (responsible for shortened ERP) and inhibited larvae.
- Can differences in the efficacies of products containing the same active ingredients be explained by differences in pharmacokinetics? Differences in solubility may result in differences in the concentrations of the active molecule in the blood stream and/or gastrointestinal fluid. Whilst blood concentrations are readily measurable, it is difficult to measure concentration in the intestinal fluid without resorting to techniques such as surgical cannulation. It may however be possible to use the concentration appearing in faeces as a proxy for levels in the gut at least for the distal parts of it.
- Is it possible to discern impacts of parasitism in terms of horse behaviour? Studies have shown how sheep walk less week by week after drenching to remove parasites, as the benefit of drenching wears off. It would be interesting to monitor the activity of horses before and after treatment to see if, as might be hypothesised from the present experiments, horses in contrast show no change in activity patterns.
- Are 2 treatments per year enough to protect the health of horses? Will younger horses be left at risk of developing disease, or performing less well if so few treatments are given?

Acknowledgements:

We would like to thank the staff at LATU for their invaluable assistance in running the study.

We would also like to acknowledge the Equine Trust for generously funding this work.

References:

Bairden K, Davies HS, Gibson NR, Hood AJO, Parker LD. Efficacy of moxidectin 2 per cent oral gel against cyathostomins, particularly third-stage inhibited larvae, in horses. *The Veterinary Record* 158, 766-8, 2006.

Bellaw JL, Krebs K, Reinemeyer CR, Norris JK, Scare JA, Pagano S, Nielsen MK. Anthelmintic therapy of equine cyathostomin nematodes – larvicidal efficacy, egg reappearance period and drug resistance. International Journal for Parasitology 48, 97-105, 2018.

Blue H. Mindful monitoring. Vetscript, 30, 42-43, 2017.

Fog P, Vigre H, Nielsen ML. Strongyle egg counts in Standardbred trotters: Are they associated with race performance? Equine Veterinary Journal 43, 89-92, 2011.

Morris LH, Colgan S, Leathwick DM, Nielsen MK. Anthelmintic efficacy of single active and combination products against commonly occurring parasites in foals. Veterinary Parasitology, 268, 46-52, 2019.

Nielsen MK, Banahan M, Kaplan RM. Importation of macrocyclic lactone resistant cyathostomins on a US thoroughbred farm. International Journal for Parasitology: Drugs and drug resistance 14, 99-104, 2020.

Reinemeyer CR, Prado JC, Nielsen MK. Comparison of the larvicidal efficacies of moxidectin or a five-day regimen of fenbendazole in horses harbouring cyathostomin populations resistant to the adulticidal dosage of fenbendazole. Veterinary Parasitology 214, 100-107, 2015.

Rosanowski SM, Bolwell CF, Scott I, Sells PD, Rogers CW. The efficacy of Ivermectin against strongyles in yearlings on Thoroughbred breeding farms in New Zealand. Veterinary Parasitology: Regional Studies and Reports 8, 70-74, 2017.

Silva RHP, de Rezende ASC, Saliba EDS, Inacio DFD, Santiago JM, Borges I, Ralston SL. The effect of deworming on apparent digestion, body weight and condition in heavily parasitised mares. Journal of Equine veterinary Science 36, 83-89.