



## Managing anthelmintic resistance in cyathostomin parasites: Investigating the benefits of *refugia*-based strategies

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### ABSTRACT

Selective anthelmintic therapy has been recommended as a sustainable strategy for cyathostomin control in horse populations for several decades. The traditional approach has been to determine strongyle fecal egg counts (FEC) for all horses, with treatment only recommended for those exceeding a predetermined threshold. The aims are to achieve a reduction of overall egg shedding, while leaving a proportion of the herd untreated, which lowers anthelmintic treatment intensity and reduces selection pressure for development of anthelmintic resistance. This study made use of the cyathostomin model to evaluate the influence of treatment strategies with between 1 and 8 yearly treatment occasions, where either 1) all horses were treated, 2) a predetermined proportion of the herd remained untreated, or 3) horses were treated if their FEC exceeded thresholds between 100 and 600 strongyle eggs per gram. Weather data representing four different climatic zones was used and three different herd age structures were compared; 1) all yearlings, 2) all mature horses 10–20 years old, and 3) a mixed age structure of 1–20 years of age. Results indicated a consistent effect of age structure, with anthelmintic resistance developing quickest in the yearling group and slowest among the mature horses. Development of anthelmintic resistance was affected by treatment intensity and selective therapy generally delayed resistance. Importantly, the results suggest that the effects of selective therapy on resistance development are likely to vary between climatic zones and herd age structures. Overall, a substantial delaying of resistance development requires that the average number of treatments administered annually across a herd of horses needs to be about two or less. However, results also indicate that an age-structured prioritisation of treatment to younger horses should still be effective. It appears that a ‘one-size-fits-all’ approach to the management of anthelmintic resistance in cyathostomins is unlikely to be optimal.

### 1. Introduction

Cyathostomin parasites are ubiquitous in grazing horses throughout the world and in modern times they constitute the most important of the equine parasites (Love et al., 1999). With widespread resistance present to the benzimidazole and tetrahydropyrimidine classes of anthelmintics (Peregrine et al., 2014), and emerging resistance to the only remaining broad-spectrum class, the macrocyclic lactones (Lyons et al., 2009, 2010; Geurden et al., 2014; Tzelos et al., 2017), chemical-based control of these parasites is becoming tenuous (Peregrine et al., 2014). Given this, the need to develop and implement changes in parasite control practices, capable of slowing the further development of resistance is obvious.

The concept of *parasite refugia* (van Wyk, 2001), which involves leaving a proportion of the worm population unexposed to anthelmintic

treatment as a source of susceptible genes, has become a corner-stone of resistance management in grazing ruminants (Leathwick and Besier, 2014; Hodgkinson et al., 2019). Both modelling (Barnes et al., 1995; Leathwick, 2012) and experimental studies (Martin et al., 1981; Waghorn et al., 2008; Leathwick et al., 2006, 2012) have demonstrated the importance of retaining unexposed (susceptible) worm genotypes *in refugia* as a means of diluting the survivors of anthelmintic treatment and slowing the development of anthelmintic resistance. Numerous approaches to achieving this end have been developed and adopted to varying degrees around the world (reviewed by Hodgkinson et al., 2019). Different approaches include; extending the interval between treatments so that susceptible parasites can develop and pass eggs onto pasture between treatments, targeting treatments only to specific classes (mobs) of animals, allowing others to remain untreated, and ‘targeted selective treatments’ where specific animals within a mob

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remain untreated on the basis of some indicator of treatment need (Leathwick and Besier, 2014; Kenyon et al., 2009). In horses, surveillance-based treatments primarily based on FEC have been widely advocated (Gomez and Georgi, 1991; Duncan and Love, 1991; Kaplan and Nielsen, 2010; Tzelos and Matthews, 2016; Nielsen et al., 2019a, 2019b; Rendle et al., 2019) but uptake by owners appears limited (Nielsen et al., 2018; Becher et al., 2018). Effectively, in most situations, all horses continue to receive multiple anthelmintic treatments each year which questions the ability of horse owners to reduce selection of resistance to these important drugs. The effectiveness of *refugia* retaining strategies at slowing the development of anthelmintic resistance has not previously been investigated in equine cyathostomins.

Here we use a newly developed model for equine cyathostomins (Leathwick et al., 2015, 2019; Nielsen et al., 2019a; Sauermann et al., 2019) to investigate different approaches to retaining *refugia* and slowing anthelmintic resistance development.

## 2. Materials and methods

### 2.1. The model

The development of the model and the incorporation of genetic mechanisms for resistance have been described elsewhere (Leathwick et al., 2015, 2019; Sauermann et al., 2019). Briefly, the model replicates the dynamics of cyathostomin parasites both on pasture and in the host. For this study the dynamics of the free-living stages was subject to climatic variables (temperature and moisture) while the parasitic stages were influenced by horse age and immunity, and exposure to infection (the ingestion of infective larvae and the presence of adult worms).

The model is capable of simulating up to three anthelmintic classes assuming a single gene mutation for each, or a lesser number of drug classes with up to three mutations involved in resistance. However, for all the simulations described here, it was assumed that resistance was the result of a single mutation in one gene, which was represented by three genotypes, a homozygous susceptible (SS), a homozygous resistant (RR) and a heterozygote (RS) with an initial resistance (R) allele frequency of  $10^{-4}$ . For simplicity, only one anthelmintic was utilized for which the efficacy was assumed to be 99%, 50% and 5% against the SS, RS and RR genotypes, respectively, of both adult worms and luminal L4, with no efficacy against the mucosal stages. The efficacies against SS stages were based on data for the efficacy of ivermectin in Klei and Torbert (1980), Klei et al. (2001), and Lyons et al. (1980). For every simulation the main output of interest was the time (in model years) until the efficacy fell below 90% for a period of at least 30 days. To avoid complications of short-term changes in allele frequency following anthelmintic treatment and to allow for the simultaneous presence of both treated and untreated horses, the calculation of efficacy was based on the R-allele frequency of the pasture L3 population as outlined elsewhere (Sauermann et al., 2019). This is referred to in subsequent sections as ‘Years to resistance’.

### 2.2. Herd age structure

The model consists of a single population of free-living parasites (on pasture) but allows for multiple parasitic worm populations (i.e. horses). Here, all simulations assumed a group of 16 horses, but three different age structures were evaluated; yearlings (0.8–2.3 years in increments of 0.1 years), mature horses (10–21.25 years in increments of 0.75 years), and a mixed age herd (1–19.75 years in increments of 1.25 years). Note that, in the model age was used as a proxy for horse immune status, which influenced several parameters in the model (Leathwick et al., 2019) including the establishment of new infection and egg count levels. Thus, the 16 horses in the simulations represented a range in immune status, between that of a naïve foal/yearling to a mature, immune competent horse. This remained unchanged, assuming

the composition of the herd stayed consistent throughout the 40-year simulation period.

### 2.3. Climate data

Climate data were chosen to represent four different Köppen-Geiger climatic zones (Menne et al., 2015); i.e., a cold humid continental climate (cold winters/mild summers, Dickinson, ND, USA), a temperate oceanic climate (mild winters/summers, Muencheberg, Germany), a humid subtropical climate (mild or no winters/hot and humid summers, high rainfall, St. Leo, FL, USA), and a hot/cold semi-arid climate (hot summers/cold winters, low rainfall, Pecos, TX, USA).

Climate data were sourced through the National Centers for Environmental Information ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)) and Deutscher Wetterdienst ([www.dwd.de](http://www.dwd.de)). For each climate zone a dataset consisting of daily min-max temperatures and rainfall covering a period of 10 years was sourced, repeated four times to allow for a total simulation period of 40 model years, and used as input data for the model.

### 2.4. Simulations

#### 2.4.1. Leaving fixed proportions untreated

In the first set of simulations, a range of scenarios were compared, which involved two, four, or eight yearly anthelmintic treatments, with differing fixed proportions of horses left untreated; i.e., 0%, 25%, and 50%. The scenario with two yearly treatment occasions was evaluated in two versions with treatments being administered either in winter and summer (December and June), or in spring and autumn (March and September). The four-treatment scenario involved anthelmintic administration in every season (March, June, September and December), while the eight yearly treatments were administered approximately every 6 weeks starting in February (Table 1). The horses to be left untreated were always the same individuals and were always the oldest (most immune) animals.

#### 2.4.2. Selective therapy

A second set of simulations evaluated the impact of anthelmintic treatments applied in a selective therapy approach where horses were only treated when their FEC exceeded a pre-determined threshold, i.e. 0, 100, 200, 300, 400, 500 and 600 strongyle eggs per gram (EPG) of faeces. A selection of 12 treatment timing scenarios were assessed with either two, three and four yearly treatments (Table 2).

#### 2.4.3. Impact on parasite populations

To better understand the dynamics between treatment number and development of anthelmintic resistance, additional outputs were summarized on the size of the third stage larval populations on pasture (L3 per m<sup>2</sup>), and the number of encysted early third stage larvae (EL3) averaged over all horses for the third simulation year.

**Table 1**

The timing (in days from January 1) of anthelmintic treatments to horses treated 8, 4 or 2 times annually in model simulations comparing the development of resistance when different fixed proportions of the herd were left untreated.

Month	Treatments per year			
	8	4	2	2
Feb	45			
Mar	90	90	90	
May	135			
June	180	180		180
Aug	225			
Sep	270	270	270	
Nov	315			
Dec	360	360		360

**Table 2**

The timing (in days from January 1) of anthelmintic treatments to horses treated two, three or four times annually in model simulations comparing the development of resistance when selective therapy was practiced with only the horses exceeding a threshold strongyle egg count being treated.

Month	Treatments/year												
	2				3				4				
Jan													30
Feb													60
Mar						90						90	
Apr	120	120				120			120				
May			150	150	150	150			150			150	
Jun							180						180
Jul						210			210	210			
Aug							240					240	
Sep	270		270						270				270
Oct		300		300							300		
Nov					330							330	
Dec													360

**3. Results**

**3.1. Leaving fixed proportions untreated**

When all horses were treated eight times a year, anthelmintic resistance invariably developed rapidly (Fig. 1). Reducing the number of annual treatments to all horses to four had some small benefit, but a sizable slowing of resistance development required only two treatments be given annually (Fig. 1). The size of these benefits varied between climate zones, being greatest in North Dakota (Fig. 1A) and least in

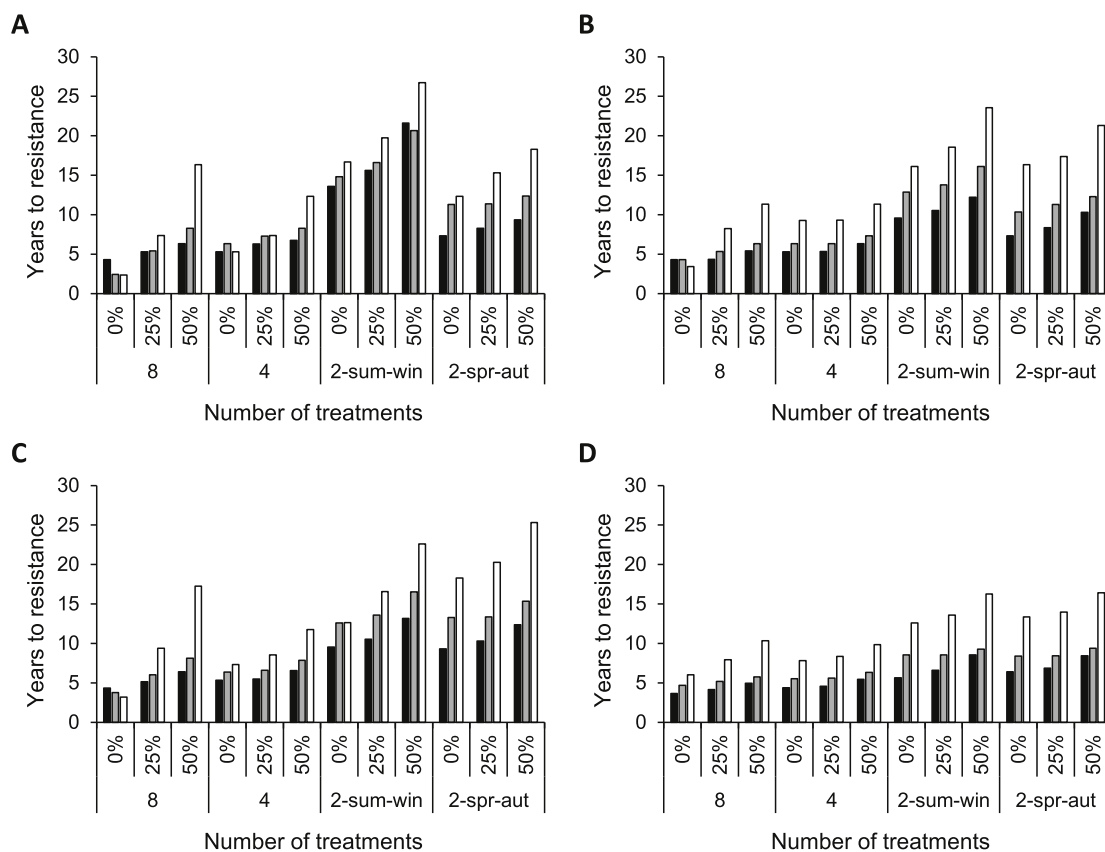
Florida (Fig. 1D). Also, when only two treatments were given annually, their timing became important as did the age structure of the herd. For two annual treatments, resistance invariably developed slower in a herd of older horses and fastest in young horses.

Leaving the same 25% or 50% of horses untreated on every occasion invariably slowed the development of resistance, but the extent to which this occurred varied between climatic zones, the time of year treatments were administered and the herd age structure (Fig. 1). Again, resistance nearly always developed slower in older horses and fastest in yearlings.

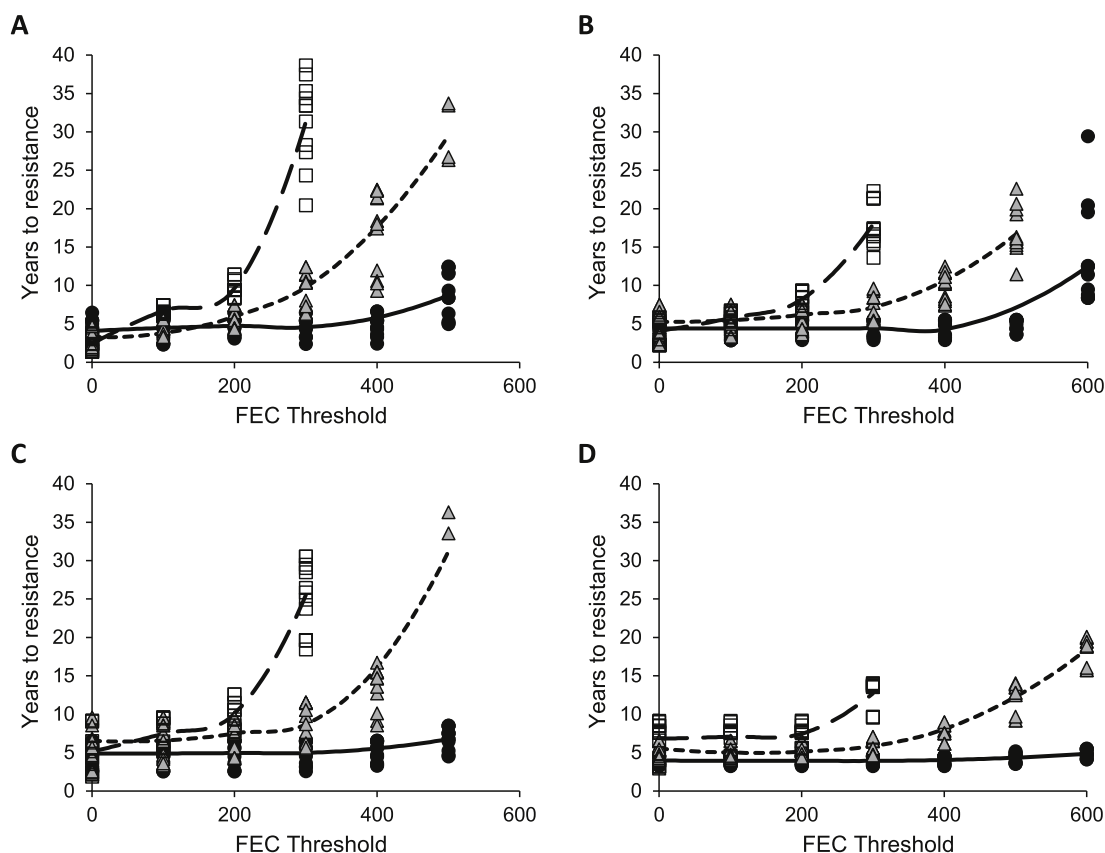
**3.2. Selective therapy**

When treatments were administered solely on the basis of faecal egg count there were noticeable differences between treatment thresholds (i.e., FEC) and between herd age structures, with some lesser differences between climates (Fig. 2). At a treatment threshold of 200 EPG or less, resistance invariably developed rapidly regardless of herd structure and climatic zone. At 300 EPG, resistance development was delayed for older horses but only to a limited extent in the mixed age herd in some climates (Fig. 2). At 400 EPG and above, resistance was delayed in older and mixed herds but only to a limited extent in yearlings and only in some climates. Differences between climates were also obvious, most markedly between ND and FL (Fig. 2 A and D).

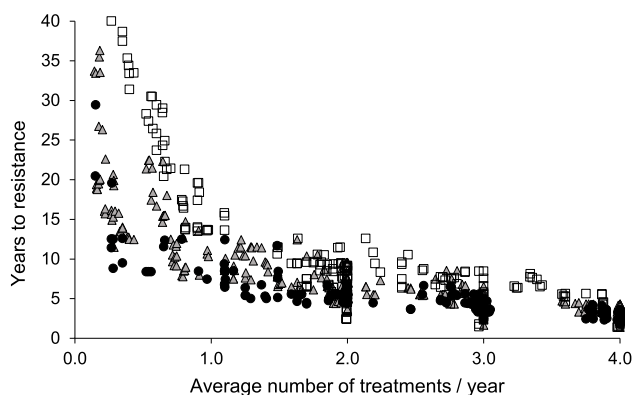
Combining all the data and averaging the number of treatments administered annually for all the different treatment scenarios showed a consistent pattern of anthelmintic resistance delay as the average number of treatments/horse declined (Fig. 3). The data showed an exponential increase in time to resistance development as the mean number of treatments administered annually declined. The divergence



**Fig. 1.** Time (in model years) for anthelmintic resistance to develop when a herd of 16 horses was treated either 8, 4 or 2 times annually with either 0, 25, or 50% of the oldest animals left untreated each time, under climate scenarios from A; North Dakota (cold, USA), B; Germany (temperate), C; Texas (hot-arid, USA) and D; Florida (hot-tropical, USA). Solid bars are all yearlings, shaded bars a mixed age herd and open bars all mature horses.



**Fig. 2.** Time (in model years) for anthelmintic resistance to develop in herds of horses managed under selective therapy regimens with a range of faecal egg count treatment thresholds (X-axis). Data presented for herds with different age structures; yearlings (solid circles), mixed (shaded triangles), and mature horses (open squares), and for four different climates; A; North Dakota (cold, USA), B; Germany (temperate), C; Texas (hot-arid, USA) and D; Florida (hot-tropical, USA).



**Fig. 3.** Time (in model years) for anthelmintic resistance to develop plotted against the mean number of anthelmintic treatments administered per horse per year. Data presented for yearlings (solid circles), mixed (shaded triangles), and mature (open squares) age structures.

between herd structures was again evident, as resistance developed faster in yearlings and slower in mature horses (Fig. 3).

### 3.3. Impact on parasite population

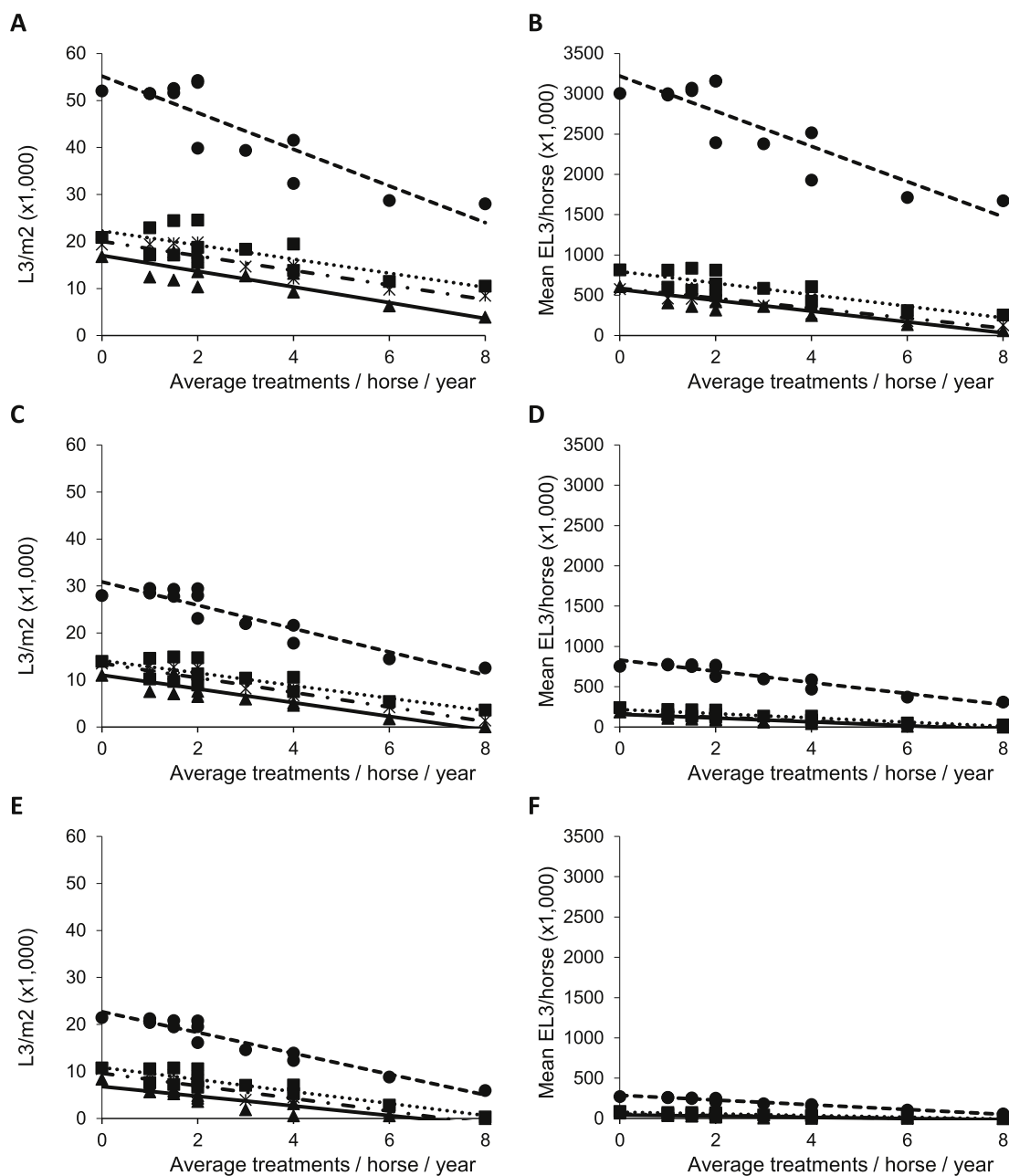
An investigation of parasite dynamics (*i.e.*, the number of infective larvae on pasture and the average number of encysted early third stage larvae in the horses in Year 3) associated with the different treatment regimens (Fig. 4) indicated a progressive increase in the size of the overall worm population as treatment frequency declined. It should be

noted that the presented EL3 values represent the average over time (1 year) across both treated and untreated horses, so they do not represent numbers in any given animal. The data also showed differences between herd structures and a separation in worm numbers between the hot-tropical (Florida) climate data and the other sites.

### 4. Discussion

Maintaining cyathostomin *parasite refugia* by leaving horses untreated has been recommended for several decades (Gomez and Georgi, 1991; Duncan and Love, 1991), but this is the first study to evaluate possible effects of this strategy. The results are consistent with those from grazing ruminants in demonstrating a benefit, in terms of slowing the development of anthelmintic resistance, of maintaining a proportion of the parasite population *in refugia* by leaving a proportion of horses untreated. But, the results also illustrate how this benefit, and potentially the optimal treatment strategy, can vary with factors such as the age structure of the herd, climate and time of year. This variation is an important finding in that it suggests that optimal treatment strategies for horses, both in terms of parasite control and management of anthelmintic resistance, may need to be tailored to climatic regions and herd structures.

Perhaps the most significant finding from this study is the indication that to achieve a significant delaying of anthelmintic resistance development, the number of annual treatments to horses needs to fall, on average, to about two/horse/year or less. When all horses were treated, reducing the number of annual treatments from eight to four had only a small effect on resistance development, regardless of herd type or climate. In fact, in the hot-tropical environment, the benefit was close to zero. Reducing the number of treatments further, to two/horse/year,



**Fig. 4.** Worm data from year 3 of model simulations where A, C and E are the mean number of L3 per  $m^2$  of pasture year, and B, D and F present the mean number of encysted early L3 larvae per horse over the year, for Florida (circles), North Dakota (triangles), Texas (asterisks) and Germany (squares). A and B are yearlings, C and D are a mixed herd, and E and F are all mature horses. Lines are least squares linear fit to the raw data.

had a much greater benefit although this showed more variability between the timing of the treatments, the climatic zones and herd structure (Fig. 1). These outcomes are likely to reflect the lifespan of adult cyathostomins within the intestinal lumen. Following an anthelmintic treatment, the contribution of eggs passed onto pasture by resistant worms surviving treatment is influenced by their lifespan. In many parasite species of sheep and cattle, the lifespan of adult worms is relatively short, *i.e.*, weeks to months (Dobson et al., 1990; Leathwick et al., 1997). In contrast, the lifespan of adult cyathostomins was estimated to be relatively long (approximately 12 months) (Leathwick et al., 2019), and so even a relatively small number of anthelmintic treatments to all horses on a farm will leave resistant worms passing eggs onto pasture for long periods. Repeated treatments within the lifespan of the adult/fecund worms will further screen this surviving population, along with any newly recruited adults which have matured

since the previous treatment, thereby gradually increasing the pool of resistant worms passing eggs onto pasture.

A similar result was evident when all treatments were administered in a selective manner based on the FEC of the horses. At low treatment thresholds (*i.e.*,  $\leq 200$  EPG) the number of treatments was sufficiently high that resistance always developed rapidly (Fig. 2). As the FEC threshold was progressively increased, fewer horses reached it and so the number of treatments declined, and resistance was delayed. Not surprisingly, this occurred at lower thresholds in older horses which by virtue of their generally lower FEC than younger horses (Boersema et al., 1996; Chapman et al., 2003) received fewer treatments than the younger herds at treatment thresholds of 300–500 EPG. An effect of climate was again evident with resistance developing more slowly in ND and fastest in FL (Fig. 2). Interestingly, in the North Dakota and Texas climates, the 600 EPG threshold for treatment was only exceeded



on a small number of occasions, such that resistance never developed even in the yearlings (Fig. 2). While, this result is undoubtedly confounded by the number of horses treated at the different threshold levels, it does highlight that the optimal FEC threshold for selective therapy is likely to be different in different climatic zones.

The relationship between treatment number and the development of anthelmintic resistance is most clearly demonstrated in Fig. 3, which summarises all the simulation results combined. This indicates that regardless of how the treatment regimens are structured, there is a consistent relationship between the average number of annual treatments per horse and the development of resistance. This suggests that regardless of whether treatments are administered strategically to all horses present or are prescribed to individual horses based on some indicator of need, the result is similar, and that the mean number of treatments needs to be less than about two/horse/year to have a large effect on the rate of resistance development. While this is realistic to achieve in adult horses, it is less so in yearlings, where the tradition is to treat as often as 6 times a year or even more (Robert et al., 2015). Yearlings are considered more susceptible to parasite infection, and thus, at greater risk for parasitic disease (Nielsen et al., 2019b). However, the current data suggests that some form of age-specific treatment structure could still produce a suitable outcome. For herds with a mixed age structure, focusing most treatments on foals and yearlings with fewer to mature horses might still achieve the desired aim of two or less treatments per horse, on average, each year. This could be particularly effective if selective therapy were to be practiced amongst the adult horses.

Encouraging a fundamental change in focus/attitude by many horse owners and veterinarians would preferably be backed by evidence for no detrimental effects as a result of reduced/no treatments to older horses. For the past decades, key opinion leaders have recommended a reduction in anthelmintic treatment intensity through the use of surveillance-based approaches to slow further development of anthelmintic resistance (Gomez and Georgi, 1991; Duncan and Love, 1991; Kaplan and Nielsen, 2010; Tzelos and Matthews, 2016; Rendle et al., 2019). But, recent questionnaire surveys have illustrated that while horse owners may be willing to reduce overall treatment intensity (Robert et al., 2015; Nielsen et al., 2018), they are reluctant to incorporate FEC monitoring in a selective treatment approach unless mandated by prescription-only legislation (Nielsen et al., 2018; Becher et al., 2018). While it should be beneficial to reduce overall treatment intensity, the lack of FEC monitoring means that the typical approach remains to always treat the entire herd, and not leave a proportion untreated regardless of their maturity or need. The results of this modelling study suggest that this needs to change with a greater focus on anthelmintic treatments to those animals at greatest risk (those under 4 years of age), which can receive the bulk of the treatments, whilst the older more mature horses are treated seldom and/or only based on demonstrable need. The results also show quite clearly that continuing to treat all or a majority of horses 4–6, or even more, times a year will inevitably lead to resistance and this will occur regardless of climatic zone or herd structure i.e. at high treatment frequencies differences between climate and herd age structure were minimal because resistance inevitable developed rapidly.

Differences between herd structures were evident in all simulations except those with high anthelmintic treatment frequencies and rapid development of resistance. In general, anthelmintic resistance was quickest to develop in the yearling herd, and slowest in the mature horse population. Although in the selective treatment scenarios this outcome was confounded with number of treatments (i.e., mature horses with lower FECs tended to receive fewer treatments), similar differences also occurred when fixed proportions of the herd were left untreated (Fig. 1). Indeed, herd differences were also evident in some climates under whole-herd treatments, although these were less obvious or consistent. This outcome suggests some difference(s) in the worm dynamics of different age classes resulting in a greater

proportional contribution of susceptible worms to subsequent worm generations when older horses are treated in contrast to yearlings. In the model, development of EL3 was slowed, and fecundity was reduced, in more immune (older) horses (Leathwick et al., 2019). Examination of detailed model outputs showed that after treatment, both adult worm burden and FEC rose faster in yearlings than in mature horses, so that at the next treatment there were more worms (and therefore more resistant genotypes) to be selected by treatment. With repeated treatments over time this resulted in a more rapid accumulation of resistant genotypes in yearlings. Evidence to support the model's behaviour can be found in studies showing that egg reappearance periods are longer in older animals and FECs often don't rise to pre-treatment levels (Smith, 1976, 1978). This modelling study is the first to indicate that this may have implications for selection of anthelmintic resistance and as such is an important finding. Further experimental investigation of this aspect of the models output would certainly seem warranted.

Effects of climate were also evident, both in terms of the development of resistance and in worm population size, most notably in the hot-tropical (Florida) environment, where the worm populations were consistently larger (Fig. 4) and resistance developed more rapidly under reduced/partial herd treatments (Figs. 1 and 2). Also, in the two climates with cold winters (North Dakota and Germany), an effect of timing of anthelmintic treatments was more pronounced with the spring-autumn scenarios leading to resistance quicker than when treatments were applied in summer and winter (Fig. 1). The explanation for these observed effects is that a climate offering complete or near year-round parasite transmission will offer greater opportunity for worms surviving treatment to produce viable offspring. In climates with defined parasite transmission seasons, resistance development will be inhibited when treatments are administered, and survivors pass eggs onto pasture, during periods of low egg/larval survival. This effect was shown in a previous modelling study where in cold and temperate climates treatments administered in winter were less selective for resistance than those administered in summer (Nielsen et al., 2019a). In contrast, resistant worms surviving treatment in more uniformly favourable year-round climates have greater opportunity to contribute resistant genotype offspring to subsequent generations, regardless of when treatments are administered.

Not surprisingly, as the number of annual treatments was reduced there was a commensurate increase in the worm populations present on pasture and in the animals (Fig. 4). To an extent this is a logical and necessary consequence of *refugia* based strategies i.e., some susceptible genotype worms must be allowed to survive and contribute to subsequent worm generations. The obvious dilemma is to balance enough unselected worms to slow the development of resistance whilst ensuring minimal impact on the health and wellbeing of the horse. It must also be remembered that non-cyathostomin parasites such as *Strongylus vulgaris*, *Anoplocephala perfoliata*, and *Parascaris* spp. are potential pathogens in horses, and should be considered in the parasite control strategy as well (Nielsen et al., 2019b). In addition, despite decades of intensive use of anthelmintics, cyathostomins persist in horses and so parasite extinction is not a practical outcome. The current modelling has shown that *refugia* based approaches have real potential to slow the development of anthelmintic resistance in cyathostomins, and so the challenge ahead is to find the balance between resistance management and optimizing equine health. A study currently underway in New Zealand is addressing the horse health consequences of reduced treatment frequencies to better understand this issue.

In summary, our results indicate that the benefits of a selective deworming approach are likely to vary between climatic zones and herd age structures. Furthermore, a FEC-based treatment threshold may need to be different for different herds to achieve optimal results. The study has highlighted the complexity of parasite control in horses of different ages and in different climates, especially those offering year-round parasite transmission. Important findings from this study are that achieving a meaningful slowing of resistance development is likely to

require a mean number of annual treatments per horse of no more than about two. While this is likely straightforward for adult horses, it would seem challenging for young horses. However, encouragingly the data suggests a mixed age herd approach with treatment intensity based on age would likely achieve the same goal. An important lesson learned from this study is that a ‘one size fits all’ recommendation for equine worm control to manage resistance development is unlikely to work for all situations. The challenge ahead will be the need to develop tailored worm control strategies for individual herds and climatic zones. Achieving this is certainly going to require considerable further work.

#### Declarations of interest

None.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jppdr.2019.08.008>.

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