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Research paper

Modelling the development of anthelmintic resistance in cyathostomin parasites: The importance of genetic and fitness parameters

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ABSTRACT

Previously described models for the free-living and parasitic phases of the cyathostomin life-cycle were combined into a single model for the complete life-cycle. The model simulates a single free-living population on pasture utilising parasite egg output from the horses and localised temperature and rainfall data to estimate infective larval density on herbage. Multiple horses of different ages are possible, each with an individualised anthelmintic treatment programme. Genotypes for anthelmintic resistance are included allowing for up to three resistance genes with 2 alleles each. Because little is known of the genetics of resistance to anthelmintics in cyathostomins, the first use of this model was to compare the effect of different assumptions regarding the inheritance of resistance on model outputs. Comparisons were made between single and two-gene inheritance, where the heterozygote survival was dominant, intermediate or recessive under treatment, and with or without a fitness disadvantage associated with the resistance mechanism. Resistance developed fastest when the heterozygotes survived anthelmintic treatment (*i.e.*, were dominant) and slowest when they did not (*i.e.*, were recessive). Resistance was slower to develop when inheritance was poly-genic compared to a single gene, and when there was a fitness cost associated with the resistance mechanism, although the latter variable was the least influential. Importantly, while these genetic factors sometimes had a large influence on the rate at which resistant genotypes built up in the model populations, their order of ranking was always the same, when different anthelmintic use strategies were compared. Therefore, the described model is a useful tool for evaluating different treatment and management strategies on their potential to select for resistance.

1. Introduction

Equine cyathostomin parasites comprise a complex of 50 species, occurring in mixed co-infections (Lichtenfels et al., 2008), which are ubiquitous in grazing horses throughout the world. They inhabit the colon and cecum of horses, where the early third larval stage (EL3) invades the mucosal barrier followed by encystment and what can be a protracted period of arrested development (Gibson, 1953). Simultaneous emergence of high numbers of encysted larvae can cause a distinct disease syndrome, known as larval cyathostominosis (Love et al., 1999), leading to a generalized severe protein-losing typhlo-colitis. This condition is associated with a 50% case-fatality rate (Reid et al., 1995).

For the past decades, cyathostomin parasites have typically been controlled by frequent application of anthelmintic treatments at fixed intervals year-round (Relf et al., 2012; Salle and Cabaret, 2015; Robert et al., 2015; Rosanowski et al., 2016). However, resistance has now been reported widely to benzimidazole and pyrantel anthelmintics (Peregrine et al., 2014), and several recent reports have suggested emerging resistance to the macrocyclic lactones, ivermectin and moxidectin, as well (Lyons et al., 2009, 2010; Peregrine et al., 2014; Bellaw et al., 2018). Understanding what management practices to change in order to slow the further development of resistance is complicated because much remains unknown regarding the biology of the parasites and the genetics underpinning resistance development (Nielsen et al., 2014). Research in sheep parasites has indicated the likely complexity involved in the genetics of resistance. For example, resistance to ivermectin in the sheep parasite *Teladorsagia circumcincta* behaves as a fully dominant character (*i.e.,* the heterozygote genotypes exhibit similar survival in the presence of drug to homozygous resistant worms), whereas resistance to moxidectin is largely recessive (Sutherland et al., 2002). In cyathostomins, beta-tubulin mutations have been associated with benzimidazole resistance (Drogemuller et al., 2004), and it seems likely that additional genetic and epi-genetic mechanisms remain as yet undiscovered (Beech et al., 2011).

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Despite these knowledge gaps, work with sheep trichostrongyle parasites has illustrated the value of computer modelling in understanding parasite biology and the development of anthelmintic resistance, and has been useful in focusing research efforts (Barnes et al., 1995; Leathwick, 2012). Recently, models have been developed for both ascarid and cyathostomin parasites of the horse (Leathwick et al., 2015a, 2016; 2017; 2019) with the cyathostomin models consisting of one for the development and survival of the free-living stages (Leathwick et al., 2015a) and a second model for the establishment and maturation of the parasitic phase (Leathwick et al., 2019). Here we combine these components into a single model for the complete cyathostomin life-cycle, before incorporating a range of possible genetic mechanisms for anthelmintic resistance. The model is then used to compare the development of resistance under different treatment strategies and evaluate the importance of the different assumptions regarding the genetics of resistance. The aim of this study was to evaluate the potential usefulness of such models for comparing treatment strategies.

2. Materials and methods

2.1. Model combination

The two models, describing the free-living and parasitic stages of cyathostomin parasites (Leathwick et al., 2015a, 2019), were combined into a single model for the complete lifecycle. The original models, which had been developed using Microsoft Excel software, were rebuilt using R (R Core Team, 2012). Briefly, the development and survival of the free-living stages, through to the infective larval stage (L3) is based on temperature, while translocation of L3 into the herbage is estimated using rainfall data. In order to capture the daily variations in temperature, this part of the model runs on an hourly time-step, using interpolation from daily max-min air temperatures to estimate hourly values. The number of L3 on the herbage and, therefore, available to be eaten, is used to calculate the daily ingestion of larvae by a grazing horse. Once L3s enter the host, the model calculates proportional establishment, arrestment of the EL3, development through the various stages and egg production by the adult worm burden. The dynamics of the parasitic phase is mainly dependent on population density and host age (as a proxy for immune status). Daily egg production adds to the free-living stages on pasture and the cycle continues. The parasitic phase of the model runs on a daily time step.

The model allows for multiple horses, of differing ages, an accompanying grazing area of 1 ha/horse and an individual anthelmintic treatment schedule can be designated for each animal. Pasture cover of available herbage was estimated using a seasonal sine wave, which varied between 1000 and 2000 kg/ha dry matter (DM). The number of infective L3 was assumed to be uniformly distributed over the available herbage and the daily ingestion by a horse was calculated assuming that each horse ingests 8 kg DM of herbage daily.

Genotypes for resistance to anthelmintic drugs were incorporated as described by Barnes and Dobson (1990) and Leathwick et al. (1995). All life-history stages were sub-divided into 27 genotypes, which allowed for simulation of resistance mechanisms involving 1, 2 or 3 independent genes with 2 alleles each. Each day, calculations for random mating of the adult parasites within each host animal, assuming a 1:1 sex ratio, determined the genotypes of the eggs excreted on that day (Leathwick et al., 1995). Following anthelmintic treatment, efficacy was calculated against each genotype of adult and luminal L4 stages by multiplying its frequency within the population by the appropriate survival rate (Table 1). For susceptible genotypes, survival values under treatment with different anthelmintics were taken from the scientific literature (Klei and Torbert, 1980; Klei et al., 2001; Lyons et al., 1980). Survival of the RR genotypes was always assumed to be high (95%), but for the current study survival of the heterozygotes was varied to investigate the effect of dominance on model output (see below).

Table 1
Anthelmintic efficacy and fitness cost in percent for each resistance mechanism and genotype used in the Cyathostomin model simulations.

Resistance mechanism	Genotype	Treatment efficacy [%]			Fitness cost [%]
		R recessive	R intermediate	R dominant	
single gene	SS	99	99	99	0
	RS	90	50	10	2.5
	RR	5	5	5	5
poly gene (two genes)	SS SS	99.0	99.0	99.0	0
	SS RS	97.8	92.9	90.5	2.5
	SS RR	90.3	90.3	90.3	5
	RS SS	97.8	92.9	90.5	2.5
	RS RS	95.0	50.0	10.0	5
	RS RR	78.2	31.1	7.5	7.5
	RR SS	90.3	90.3	90.3	5
	RR RS	78.2	31.1	7.5	7.5
	RR RR	5.0	5.0	5.0	10

2.2. Simulation scenarios

For all simulations evaluated here, a herd of 10 horses with an evenly distributed range of ages was used. In this model horse age is used as a proxy for immune status to reflect the fact that older horses have greater experience of parasite exposure and immune competence, and so selecting a range of ages, between that of a relatively naïve yearling to that of a mature immune competent horse, reflects a situation common on many horse properties. The immune status of the horses remained unchanged during the simulation period to maintain a consistent herd composition.

Simulations were conducted to compare the effect on the development of resistance of single and poly genetic (2 gene) inheritance, when the heterozygotes were either dominant, intermediate or recessive under treatment and whether or not the resistance gene(s) carried a fitness cost. For each resistance gene there were three genotypes, a homozygous susceptible (SS), a heterozygote (RS) and a homozygous resistant (RR), resulting in three genotypes for the single gene model and nine for the polygene model (Table 1). The effective dominance of the heterozygotes was taken as the ability to survive treatment with anthelmintic *i.e.,* when the RS is dominant its survival under treatment will be similar to the RR genotype, while when recessive it will be similar to the SS genotype and intermediate equates to 50% survival when treated (Table 1). The possibility of a negative pleiotropic effect of an R-allele associated to parasite fitness was applied as either a 0% or 2.5% reduction in fecundity for each R-allele present in a genotype (Table 1). The starting frequencies for the R alleles were set to 0.0001 for the single gene and 0.01 for the polygene simulations. These values were chosen to give an equivalent efficacy (98%) of treatment at the commencement of each simulation regardless of whether single or polygenic inheritance was assumed.

All possible permutations of the above were compared under four different anthelmintic treatment strategies (Table 2); *i.e.*, 1) treat every horse six times per year (Days 60, 120, 180, 240, 300, 360, where Day 1 = January 1); 2) treat every horse three times per year (Days 120, 240, 360) and two strategies using the same 3 treatment dates as for 2), but

Table 2
Treatment strategies compared using the horse cyathostomin model indicating the time of treatment and the percentage of horses in the herd remaining untreated.

ID	Treatments per year	Untreated horses	Treatment times
Trt1	6	0%	Feb, Apr, Jun, Aug, Oct, Dec
Trt2	3	0%	Feb, Jun, Oct
Trt3	3	30%	Feb, Jun, Oct
Trt4	3	70%	Feb, Jun, Oct

leaving either 30% (Trt3) or 70% (Trt4) of the oldest horses untreated. For all treatments, anthelmintic efficacy was applied to luminal L4 and adult worms with no persistent activity, *i.e.,* the treatment was only effective on the day of treatment.

The comparisons were run using weather data from two contrasting environments. Climate data sets spanning 10 years were acquired from National Centers for Environmental Information (www.ncdc.noaa.gov) and Deutscher Wetterdienst (www.dwd.de) representing two different Köppen-Geiger climate conditions *i.e.*, a temperate oceanic climate (Muencheberg, Germany) and humid subtropical climate (Washington, Georgia, USA). Each data set was repeated four times to enable each simulation to run for 40 years.

2.3. Comparing treatment strategies

For every simulation the output of interest was the time (in model years) until efficacy, calculated using the R allele frequency in the L3 population on pasture, fell below 90% for a period of at least 30 days. Calculating R allele frequency for the pasture larval population allowed for a measure of change in anthelmintic efficacy, which avoided the rapid fluctuations associated with short-term changes in the parasite populations within the hosts following treatment. Furthermore, this approach allowed for having untreated horses within the herd in that it is difficult to follow long-term changes in anthelmintic efficacy when some horses are treated regularly, and others are not. This enabled a comparison of the effect of the different genetic variables on the rate at which resistance alleles built up in the model worm population when horses with low faecal egg count (FEC) were not treated. In order to compare the different anthelmintic treatment strategies, independent of the genetics of resistance, the four strategies were ranked (1–4) within each set of genetic assumptions on the time required for efficacy to fall below 90%. In situations where the efficacy did not fall below 90% within the 40 year simulation period, ranking was based on the mean efficacy over the last 30 days of the simulation.

3. Results

The model produces output for all life-history stages, a single population on pasture and a separate population for every horse within the simulation (Fig. 1). Output also tracks the different genotypes showing the build-up of resistant genotypes under the anthelmintic treatment regimens evaluated (Fig. 2).

The number of genes involved, their dominance under treatment and a fitness cost associated with the resistance mechanism all influenced the development of resistance in the model (Fig. 3). Resistance developed faster when it was the result of a single gene rather than poly-genic, and when the heterozygote(s) survived anthelmintic treatment (*i.e.,* the RS genotype was effectively dominant). The differences between genetic mechanisms resulted in > 10-fold differences in the time for efficacy to fall below 90%, emphasising how important these factors can be in resistance development. However, of the different assumptions tested it would appear that the survival of the heterozygote (s) under treatment had the greatest effect on the rate at which resistance developed in the populations, while a fitness cost associated with resistance had the least effect (Fig. 3). Of note was that in a small number of simulations, when resistance was assumed to be recessive and a fitness cost was applied, the resistant allele frequency fell below the initial value during the simulation period (results not shown) *i.e.*, the resistance gene(s) became rarer in the population indicating that the fitness cost was more influential than the benefit gained from the resistance gene(s).

Despite the large differences in the rate at which resistance developed under the different genetic mechanisms, when the treatment strategies were ranked in order of which ones selected for resistance the fastest, the results were invariably the same (Table 3). Treating all horses six times annually always resulted in resistance developing faster than the other treatment strategies. In contrast, when 70% of horses remained untreated, resistance was slowest to develop and only developed, within the 40-year simulation period, when it was not recessively inherited (Fig. 3).

4. Discussion

We have constructed the first computer model simulating the entire cyathostomin life cycle, including both free-living and parasitic stages. The model allows for multi-horse herds with individualised anthelmintic treatment strategies, different climatic conditions and the development of anthelmintic resistance. Model outputs include faecal egg counts as well as counts of any life-cycle stage of interest, and it allows evaluation of the time for resistance to develop under a chosen

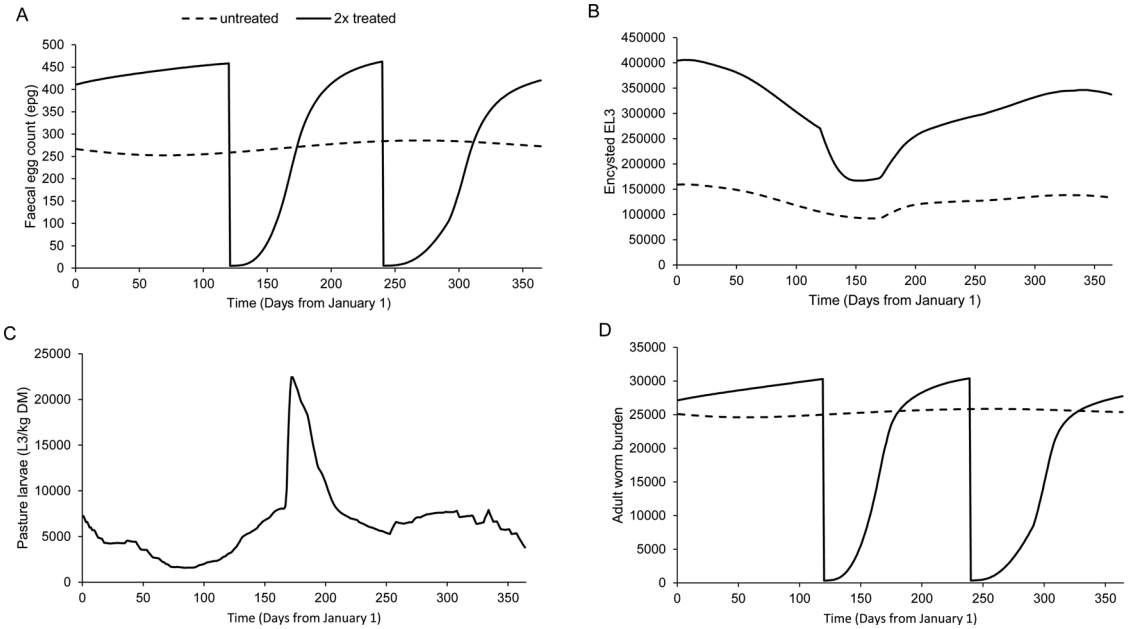

Fig. 1. Model output for faecal nematode egg count (A), encysted early L3 (B), pasture larvae contamination (C) and adult worm burden (D) when two horses graze a pasture in Georgia (USA), of which one horse is 8 years old and is treated with anthelmintic twice a year and the other is 16 years old and is never treated.

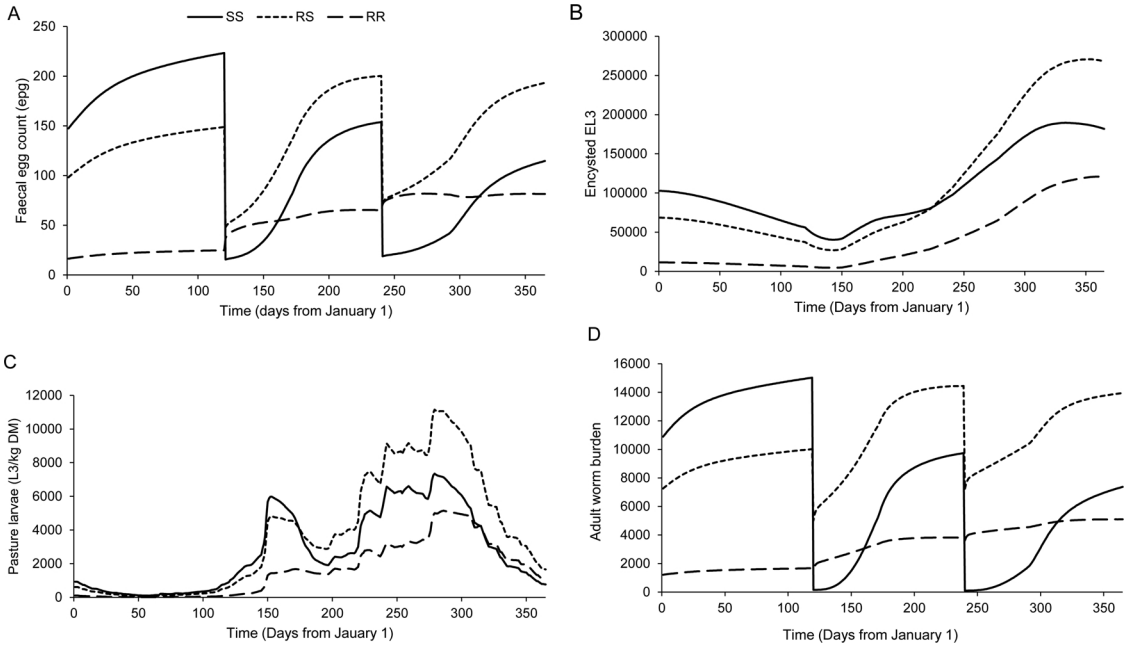

Fig. 2. Model output for faecal egg count (A), encysted early L3 (B), pasture larvae contamination (C) and adult worm burden (D) when an 8-year old horse grazes a pasture in Georgia (USA), is treated with anthelmintic twice a year, with output divided into different genotypes for anthelmintic resistance. Resistance allele frequency is set at 0.25 with dominance being intermediate.

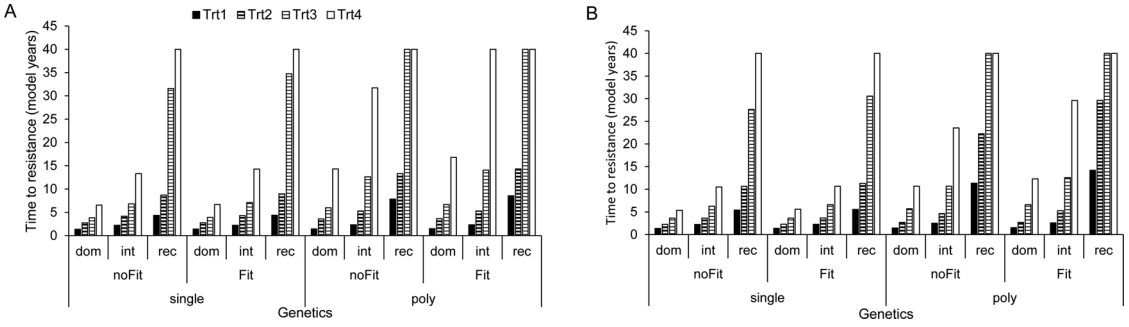

Fig. 3. Simulation results of the time to anthelmintic resistance in a cyathostome population for 4 treatment strategies under various genetic resistance mechanisms. Time to resistance is calculated as the time for the efficacy, calculated on genotypes in the free-living third-stage larvae population, to fall below 90% for at least 30 days. Shorter times indicate faster development of resistance. Trt 1 = 6 treatments per year every 60 days, Trt 2 = 3 treatments per year every 120 days, Trt 3 = same as Trt 2 but leaving 30% of the horses untreated, Trt 4 = same as Trt 2 but leaving 70% of the horses untreated. A = Georgia (USA) and B = Muencheberg (Germany).

treatment scenario.

Model outputs appear reasonable, given that there are no complete field data sets against which to compare. Burdens of luminal stages are consistent with published data, but the variation in counts between different published works makes comparison with model output difficult, as does the very high variability in worm count data within these studies (Bucknell et al., 1995; Klei and Chapman, 1999; Nielsen et al., 2010). While acknowledging that serial slaughter data for horses is no small undertaking, it is perhaps surprising there appears to be no published data relating FECs over time with pasture larval populations for horses, especially under different anthelmintic treatment regimes. As reduction of pasture strongyle infectivity has been identified as the main goal with current equine parasite control recommendations (Nielsen et al., 2013), it would be valuable if future studies aimed at evaluating pasture larval counts in response to various treatment regimens. This type of data could then be used to validate, and if necessary, further refine the model.

Because little is known of the genetics or mechanisms of resistance in any equine parasites, it was an important first step to determine whether the model could be useful for evaluating treatment and management strategies on their potential to select for resistance in cyathostomins. Hence, this first use of the model was to compare a range of different assumptions for the genetics of resistance. Outputs indicate that resistance will develop fastest when it behaves as a dominant trait *i.e.*, survival of the heterozygote is high, similar to that of the homozygous resistant genotype, in the presence of drug. This is consistent with findings from earlier studies (Gettinby, 1989; Smith, 1990; Barnes et al., 1995). This also is an important practical finding because heterozygote survival is one variable that can potentially be manipulated. Studies of anthelmintic pharmacology have shown that actives delivered by different routes can result in large differences in the concentration of active reaching the target parasites (Bogan and McKellar, 1988; Gokbulut et al., 2010; Lloberas et al., 2012) and that this can result in substantial differences in efficacy against resistant worm genotypes (Gopal et al., 2001; Leathwick and Miller, 2013). Differences in the concentrations of active, which allow differential survival of resistant worm genotypes, can lead to a more rapid development of resistance (Leathwick and Luo, 2017). Several studies in horses displayed similar results (Gokbulut et al., 2010; Saumell et al., 2017) and so it seems reasonable that route of administration is a

Table 3
Ranking of anthelmintic treatment strategies under a range of assumptions for the genetics of resistance, using climate data for Muencheberg (Germany) and Georgia (USA), where treatments are; 1) treat every horse six times per year (Trt1); 2) treat every horse three times per year (Trt2), and two strategies using the same treatment dates as for 2), but leaving either 30% (Trt3) or 70% (Trt4) of the oldest horses untreated.

Climate	Genetics	Fitness cost	Dominance	Trt1	Trt2	Trt3	Trt4
Muencheberg (Germany)	single	none	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
		2.5%	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
	poly	none	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
		2.5%	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
Georgia (USA)	single	none	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
		2.5%	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
	poly	none	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1
		2.5%	dom	4	3	2	1
			int	4	3	2	1
			rec	4	3	2	1

variable worthy of consideration when deciding on strategies to slow the development of resistance.

Studies in ruminant nematode parasites have shown considerable complexity in the genetics of resistance associated with different parasite species and anthelmintics, with indications of both single and polygenic mechanisms and inheritance being recessive, intermediate and dominant under treatment (Sangster et al., 1998; Martin and McKenzie, 1990; Sutherland et al., 2002; Doyle et al., 2019). The limited data available on equine parasites suggests that similar mechanisms and diversity are likely (Blackhall et al., 2011; Kaschny et al., 2015) and so no easy generalisations are possible at this time. Regardless, the number of genes is not a variable that is likely to be open to easy manipulation.

In these model outputs, the least influential of the assumptions was that there is a fitness cost associated with the resistance mechanism. However, this may be expected to an extent because these simulations all involved continuous use of a single drug. The effect of a fitness disadvantage in slowing the emergence of anthelmintic resistance is more likely to be seen when different classes of drugs are used in some form of rotation, or even more so when drugs are used in combination (Leathwick, 2013). In a situation where a single active is used continuously, the advantage conferred on the resistant genotypes is usually far in excess of any counter-selection applied by a fitness disadvantage, as was the case here, except when resistance was recessive. Fitness costs have been associated with resistant nematode isolates in some cases, and these have usually been small (Donald et al., 1980; Waller et al., 1989), but not always (Maingi et al., 1990). However, measuring these costs is made confusing by the potential for co-selection of both resistance and fitness genes as resistance develops in specific populations (Martin et al., 1988; Prichard, 1990) and so the fitness costs associated with resistant genotypes when these are rare in populations is largely unknown. Here, we have used a fitness cost equivalent to a 5–10% disadvantage in RR genotypes which have been shown in previous modelling studies capable of delaying the emergence of resistance (Leathwick, 2013), but, there is no real evidence as to whether this is a realistic value or not. Regardless, evidence for the presence of fitness costs associated with resistant isolates of sheep parasites has been shown in the field, where reversion toward susceptibility was documented when resistance management strategies, including careful refugia management, were implemented (Leathwick et al., 2015b). To date no studies have investigated the possibility of fitness costs associated with resistance in cyathostomins.

Possibly the most important finding from this study is the fact that despite the variations in time for resistance to develop under the different assumptions for the genetics of resistance, ranking the different strategies produced consistent results between them. *I.e.*, when comparing the treatment scenarios on which selected the fastest or slowest for resistance, the best scenario was always the best and the worst was always the worst (Barnes et al., 1995). This is important because while anthelmintic resistance is common and widespread in the cyathostomins (Peregrine et al., 2014) little is known of its genetic basis (Nielsen et al., 2014). As illustrated herein, models can be used to investigate resistance management without knowing the specifics of the resistance mechanisms. It is almost certain that all of the current anthelmintics would have failed long before all genetic mechanisms were identified, which would make the model a pointless exercise. Cyathostomin parasites pose some unique practical challenges to those wishing to study the genetics of resistance, not least of which are the complex of species, and the presence of a protracted period of larval encystment with burdens, which cannot easily be removed once they have established. Fortunately, the current outputs suggest that as long as the model is used to compare different treatment strategies, and not attempt to predict the timing of resistance development, then the model outputs are likely to be valid and useful.

Four different anthelmintic use strategies were compared in this study, with the primary purpose of allowing their ranking on the rate at which resistance develops under the different genetic models. The consistent and often large differences between these strategies is encouraging as it implies considerable potential to delay the further development of resistance in these parasites. However, proper investigation of different potential management strategies will require further work, including consideration of different climatic zones and seasonality, the age structure of herds and the importance of maintaining control of other important parasites. From the current study, it appears that this model is a potentially useful tool in balancing all these factors in order to manage anthelmintic resistance in these important parasites of the horse.

Conflict of interest statement

The authors declare no conflicts of interest.

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References

Barnes, E.H., Dobson, R.J., 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: computer model to simulate grazing systems and the evolution of anthelmintic resistance. Int. J. Parasitol. 20, 823–831.

Barnes, E.H., Dobson, R.J., Barger, I.A., 1995. Worm control and anthelmintic resistance: adventures with a model. Parasitol. Today (Regul. Ed.) 11, 56–63.

Beech, R.N., Skuce, P., Bartley, D.J., Martin, R.J., Prichard, R.K., Gilleard, J.S., 2011. Anthelmintic resistance: markers for resistance or susceptibility? Parasitology 138, 160–174.

Bellaw, J.L., Krebs, K., Reinemeyer, C.R., Norris, J.K., Scare, J.A., Pagano, S., Nielsen, M.K., 2018. Anthelmintic therapy of equine cyathostomin nematodes – larvicidal efficacy, egg reappearance period, and drug resistance. Int. J. Parasitol. 48, 97–105.
Blackhall, W.J., Kuzmina, T., von Samson-Himmelstjerna, G., 2011. B-Tubulin genotypes in six species of cyathostomins from anthelmintic-naïve Przewalski and benzimidazole-resistant brood horses in Ukraine. Parasitol. Res. 109, 1199–1203.
Bogan, J.A., McKellar, Q.A., 1988. The pharmacokinetics of ivermectin in sheep and cattle. J. Vet. Pharmacol. Therap. 11, 260–268.
Bucknell, D.G., Gasser, R.B., Beveridge, I., 1995. The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. Int. J. Parasitol. 25, 711–724.
Donald, A.D., Waller, P.J., Dobson, R.J., Axelsen, A., 1980. The effect of selection with levamisole on benzimidazole resistance in *Ostertagia* spp. of sheep. Int. J. Parasitol. 10, 381–389.
Doyle, S.R., Illingworth, C.J.R., Laing, R., Bartley, D.J., Redman, E., Martinelli, A., Holroyd, N., Morrison, A., Rezansoff, A., Tracey, A., Devaney, E., Berriman, M., Sargison, N., Cotton, J.A., Gilleard, J.S., 2019. Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, Haemonchus contortus. BMC Genomics 20, 218.
Drogemuller, M., Schnieder, T., von Samson-Himmelstjerna, G., 2004. Beta-tubulin complementary DNA sequence variations observed between cyathostomins from benzimidazole-susceptible and -resistant populations. J. Parasitol. 90, 868–870.
Gettinby, G., 1989. Computational veterinary parasitology with an application to chemical resistance. Vet. Parasitol. 32, 57–72.
Gibson, T.E., 1953. The effect of repeated anthelmintic treatment with phenothiazine on fecal egg counts of housed horses, with some observations on the life cycle of *Trichonema* spp. in the horse. J. Helminthol. 27, 29–40.
Gokbulut, C., Cirak, V.Y., Senlik, B., Aksit, D., Durmaz, M., McKellar, Q.A., 2010. Comparative plasma disposition, bioavailability and efficacy of ivermectin following oral and pour-on administration in horses. Vet. Parasitol. 170, 120–126.
Gopal, R.M., West, D.M., Pomroy, W.E., 2001. The difference in efficacy of ivermectin oral, moxidectin oral and moxidectin injectable formulations against an ivermectin resistant strain of *Trichostrongylus colubriformis* in sheep. N. Z. Vet. J. 49, 133–137.
Kaschny, M., Demeler, J., Janssen, I.J.I., Kuzmina, T.A., Besognet, B., Kanellos, T., Kerboeuf, D., von Samson-Himmelstjerna, G., Krűcken, J., 2015. Macrocyclic lactones differ in interaction with recombinant p-glycoprotein 9 of the parasitic nematode *Cylicocylus elongatus* and ketoconazole in a yeast growth assay. PLOS path 11, e1004781.
Klei, T.R., Torbert, B.J., 1980. Efficacy of ivermectin (22,23-dihydroavermectin B1) against gastrointestinal parasites in ponies. Am. J. Vet. Res. 41, 1747–1750.
Klei, T.R., Chapman, M.R., 1999. Immunity in equine cyathostome infections. Vet. Parasitol. 85, 123–136.
Klei, T.R., Rehbein, S., Visser, M., Langholff, W.K., Chapman, M.R., French, D.D., Hanson, P., 2001. Re-evaluation of ivermectin efficacy against equine gastrointestinal parasites. Vet. Parasitol. 98, 315–320.
Leathwick, D.M., 2012. Modelling the benefits of a new class of anthelmintic in combination. Vet. Parasitol. 186, 93–100.
Leathwick, D.M., 2013. Managing anthelmintic resistance – parasite fitness, drug use strategy and the potential for reversion towards susceptibility. Vet. Parasitol. 198, 145–153.
Leathwick, D.M., Luo, D., 2017. Managing anthelmintic resistance - Variability in the dose of drug reaching the target worms influences selection for resistance? Vet. Parasitol. 243, 29–35.
Leathwick, D.M., Miller, C.M., 2013. Efficacy of oral, injectable and pour-on formulations of moxidectin against gastrointestinal nematodes in cattle in New Zealand. Vet. Parasitol. 191, 293–300.
Leathwick, D.M., Vlassoff, A., Barlow, N.D., 1995. A model for nematodiasis in New Zealand lambs: the effect of drenching regime and grazing management on the development of anthelmintic resistance. Int. J. Parasitol. 25, 1479–1490.
Leathwick, D.M., Donecker, J.M., Nielsen, M.K., 2015a. A model for the dynamics of the free-living stages of equine cyathostomins. Vet. Parasitol. 209, 210–220.
Leathwick, D.M., Ganesh, S., Waghorn, T.S., 2015b. Evidence for reversion towards susceptibility in *Teladorsagia circumcincta* in response to resistance management programmes. Int. J. Parasitol. –Drugs and Drug Res. 5, 9–15.
Leathwick, D.M., Sauermann, C.W., Donecker, J., Nielsen, M.K., 2016. A model for the development and growth of the parasitic stages of *Parascaris* spp. in the horse. Vet. Parasitol. 228, 108–115.
Leathwick, D.M., Sauermann, C.W., Geurden, T., Nielsen, M.K., 2017. Managing anthelmintic resistance in *Parascaris* spp.: a modelling exercise. Vet. Parasitol. 240, 75–81.
Leathwick, D.M., Sauermann, C.W., Reinemeyer, C.R., Nielsen, M.K., 2019. A model for the dynamics of the parasitic stages of equine cyathostomins. Vet. Parasitol. 268, 53–60.
Lichtenfels, J.R., Kharchenko, V.A., Dvojnos, G.M., 2008. Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Vet. Parasitol. 156, 4–161.
Lloberas, M., Alvarez, L., Entrocasso, C., Virkel, G., Lanusse, C., Lifschitz, A., 2012. Measurement of ivermectin concentrations in target worms and host gastrointestinal tissues: influence of the route of administration on the activity against resistant *Haemonchus contortus* in lambs. Exp. Parasitol. 131, 304–309.
Love, S., Murphy, D., Mellor, D., 1999. Pathogenicity of cyathostome infection. Vet. Parasitol. 85, 113–122.
Lyons, E.T., Drudge, J.H., Tolliver, S.C., 1980. Antiparasitic activity of ivermectin in critical tests in equids. Am. J. Vet. Res. 41, 2069–2072.
Lyons, E.T., Tolliver, S.C., Collins, S.S., 2009. Probable reason why small strongyle EPG counts are returning “early” after ivermectin treatment of horses on a farm in Central Kentucky. Parasitol. Res. 104, 569–574.
Lyons, E.T., Tolliver, S.C., Kuzmina, T.A., Collins, S.S., 2010. Critical tests evaluating efficacy of moxidectin against small strongyles in horses from a herd for which reduced activity had been found in field tests in Central Kentucky. Parasitol. Res. 107, 1495–1498.
Maingi, N., Scott, M.E., Prichard, R.K., 1990. Effect of selection pressure for thiabendazole resistanceon fitness of *Haemonchus contortus* in sheep. Parasitol. 100, 327–335.
Martin, P.J., McKenzie, J.A., 1990. Levamisole resistance in *Trichostrongylus colubriformis*: a sex-linked recessive character. Int. J. Parasitol. 20, 867–872.
Martin, P.J., Anderson, T.H., Brown, T.H., Miller, D.W., 1988. Changes in resistance of *Ostertagia*spp. to thiabendazole following natural selection or treatment with levamisole. Int. J. Parasitol. 18, 333–340.
Nielsen, M.K., Baptiste, K.E., Tolliver, S.C., Collins, S.S., Lyons, E.T., 2010. Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. Vet. Parasitol. 174, 77–84.
Nielsen, M.K., Reinemeyer, C.R., Sellon, D.C., 2013. Nematodes. Equine Infectious Diseases, Second Edition. pp. 475–489 475-489.e474.
Nielsen, M.K., Reinemeyer, C.R., Donecker, J.M., Leathwick, D.M., Marchiondo, A.A., Kaplan, R.M., 2014. Anthelmintic resistance in equine parasites-Current evidence and knowledge gaps. Vet. Parasitol. 204, 55–63.
Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic resistance in important parasites of horses: does it really matter? Vet. Parasitol. 201, 1–8.
Prichard, R.K., 1990. Anthelmintic resistance in nematodes: extent, recent understanding and future directions for control and research. Int. J. Parasitol. 20, 515–523.
R Core Team, 2012. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Reid, S.W., Mair, T.S., Hillyer, M.H., Love, S., 1995. Epidemiological risk factors associated with a diagnosis of clinical cyathostominosis in the horse. Equine Vet. J. 27, 127–130.
Relf, V.E., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2012. A questionnaire study on parasite control practices on UK breeding Thoroughbred studs. Equine Vet. J. 44, 466–471.
Robert, M., Hu, W., Nielsen, M.K., Stowe, C.J., 2015. Attitudes towards implementation of surveillance-based parasite control on Kentucky Thoroughbred farms – current strategies, awareness, and willingness-to-pay. Equine Vet. J. 47, 694–700.
Rosanowski, S.M., Scott, I., Sells, P.D., Rogers, C.W., Bolwell, C.F., 2016. Cross-sectional survey of parasite control practices on Thoroughbred and Standardbred training yards in New Zealand. Equine Vet. J. 48, 387–393.
Salle, G., Cabaret, J., 2015. A survey on parasite management by equine veterinarians highlights the need for a regulation change. Vet. Rec. Open 2, e000104.
Sangster, N.C., Redwin, J.M., Bjorn, H., 1998. Inheritance of levamisole and benzimidazole resistance in an isolate of Haemonchus contortus. Int. J. Parasitol. 28, 503–510.
Saumell, C., Lifschitz, A., Baroni, R., Fusé, L., Bistoletti, M., Sagües, F., Bruno, S., Alvarez, G., Lanusse, C., Alvarez, L., 2017. The route of administration drastically affects ivermectin activity against small strongyles in horses. Vet. Parasitol. 236, 62–67.
Smith, G., 1990. A mathematical model for the evolution of anthelmintic resistance in a direct life cycle nematode parasite. Int. J. Parasitol. 20, 913–921.
Sutherland, I.A., Leathwick, D.M., Moen, I.C., Bisset, S.A., 2002. Resistance to therapeutic treatment with macrocyclic lactone anthelmintics in Ostertagia circumcincta. Vet. Parasitol. 109, 91–99.
Waller, P.J., Donald, A.D., Dobson, R.J., Lacey, E., Hennesey, D.R., Allerton, G.R., Prichard, R.K., 1989. Changes in anthelmintic resistance status of Haemonchus contortus and *Trichostrongylus colubriformis* exposed to different anthelmintic selection pressures in grazing sheep. Int. J. Parasitol. 19, 99–110.