Veterinary Parasitology 269 (2019) 7–12

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

The effect of climate, season, and treatment intensity on anthelmintic resistance in cyathostomins: A modelling exercise

Martin K. Nielsen[a,*], Christian W. Sauermann[b], Dave M. Leathwick[b]

[a] M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA
[b] AgResearch Grasslands, Private Bag 11008, Palmerston North, 4442, New Zealand

ARTICLE INFO

Keywords:
Cyathostomin
Simulation
Anthelmintic resistance
Refugia
Climate
Selective therapy

ABSTRACT

Anthelmintic resistance is widespread in equine cyathostomin populations across the world, and with no new anthelmintic drug classes in the pharmaceutical pipeline, the equine industry is forced to abandon traditional parasite control regimens. Current recommendations aim at reducing treatment intensity and identifying high strongylid egg shedders in a targeted treatment approach. But, virtually nothing is known about the effectiveness of these recommendations, nor their applicability to different climatic regions, making it challenging to tailor sustainable recommendations for equine parasite control. This study made use of a computer model of the entire cyathostomin life-cycle to evaluate the influence of climate and seasonality on the development of anthelmintic resistance in cyathostomin parasites. Furthermore, the study evaluated the impact of recommended programs involving selective anthelmintic therapy on delaying anthelmintic resistance development. All simulations evaluated the use of a single anthelmintic (*i.e.,* ivermectin) over the course of 40 model years. The study made use of weather station data representing four different climatic zones: a cold humid continental climate, a temperate oceanic climate, a cold semi-arid climate, and a humid subtropical climate. Initially, the impact of time of the year was evaluated when a single anthelmintic treatment was administered once a year in any of the twelve months. The next simulations evaluated the impact of treatment intensities varying between 2 and 6 treatments per year. And finally, we evaluated treatment schedules consisting of a combination of strategic treatments administered to all horses and additional treatments administered to horses exceeding a pre-determined fecal egg count threshold. Month of treatment had a large effect on resistance development in colder climates, but little or no impact in subtropical and tropical climates. Resistance development was affected by treatment intensity, but was also strongly affected by climate. Selective therapy delayed resistance development in all modelled scenarios, but, again, this effect was climate dependent with the largest delays observed in the colder climates. This study is the first to demonstrate the value of cyathostomin parasite refugia in managing anthelmintic resistance, and also that climate and seasonality are important. This modelling exercise has allowed an illustration of concepts believed to play important roles in anthelmintic resistance in equine cyathostomins, but has also identified knowledge gaps and new questions to address in future studies.

1. Introduction

Anthelmintic resistance in equine cyathostomin parasites has become extremely common throughout the world, with reports of resistance to all three of the currently available anthelmintic classes (Peregrine et al., 2014). Of most concern are recent reports of reductions of cyathostomin egg reappearance periods (ERP) following treatment with ivermectin or moxidectin (Lyons et al., 2008, 2011; Geurden et al., 2014; Tzelos et al., 2017), which in light of widespread resistance to the other classes have become the mainstay of cyathostomin control throughout the world. A shortening of the ERP is likely to be the first indication of developing anthelmintic resistance (Sangster, 1999), a hypothesis supported by recent studies in which shortened ERPs following ivermectin or moxidectin treatment were associated with survival of luminal, fourth-stage larvae (Lyons et al., 2009, 2010; Bellaw et al., 2018).

Cyathostomins infect virtually every grazing horse across the planet and are responsible for a severe disease syndrome known as larval cyathostominosis (Love et al., 1999). Because of the increasing levels of anthelmintic resistance, numerous authors have emphasised the need to move away from the traditional frequent calendar-based treatment approach to more surveillance-based treatment strategies in order to

* Corresponding author.
E-mail address: martin.nielsen@uky.edu (M.K. Nielsen).

https://doi.org/10.1016/j.vetpar.2019.04.003
Received 5 February 2019; Received in revised form 1 April 2019; Accepted 6 April 2019

preserve the efficacy of the currently available anthelmintic compounds (Herd, 1990; Duncan and Love, 1991; Gomez and Georgi, 1991; Kaplan and Nielsen, 2010). Despite these recommendations, strategies commonly employed for parasite control in domestic horses continue to involve frequent whole-herd treatments with broad spectrum anthelmintics applied at regular intervals year-round (Relf et al., 2012; Salle and Cabaret, 2015; Robert et al., 2015; Rosanowski et al., 2016; Becher et al., 2018; Nielsen et al., 2018). While it is commonly believed that such treatment regimens are largely responsible for the reduced anthelmintic efficacy levels reported today, it remains unknown how effective the recommended surveillance-based strategies are likely to be in reducing further development of resistance.

We recently developed a computer model which captures the entire cyathostomin lifecycle, covering the free-living stages in the environment (Leathwick et al., 2015) as well as the parasitic stages within the horse (Leathwick et al., 2019). Subsequently, this model was expanded to include anthelmintic treatments and genetic mechanisms representing anthelmintic resistance (Sauermann et al., unpublished data). Here, we make use of this model to evaluate the effect of the time of year on resistance development, when treatments are administered in different climatic zones. Furthermore, we examine commonly employed parasite treatment strategies, as well as some of the recommended alternatives, with special emphasis on the development of anthelmintic resistance.

2. Materials and methods

2.1. The model

The derivation of the model for the dynamics of cyathostomins in horses, and the incorporation of genetics for anthelmintic resistance, have been described previously (Leathwick et al., 2015, 2019; Sauermann et al., unpublished data). Briefly, the model replicates the dynamics of cyathostomin life-history stages both on pasture and in the host. Eggs passed onto pasture in faeces from infected horses develop through two pre-infective stages (L1/L2) to become infective third stage larvae (L3) which, in the presence of moisture, then migrate onto herbage to become available for ingestion by a host. Development and survival rates for these stages are determined by temperature and moisture. The performance of the free-living stages model was tested against published data sets (Leathwick et al., 2015). Once ingested, a proportion of L3 migrate into the tissues of the large intestine and encyst as early L3s (EL3). These undergo a variable period of arrested development which is controlled by the presence of adult worms in the lumen and the host's recent exposure to ingested L3, before resuming development as late L3s (LL3s) and moulting to the fourth larval stage (L4) in the mucosa. Following migration into the intestinal lumen, further advancement to the adult stage is restricted by the presence of adult worms and the L4s die off over time if they are prevented from developing to the adult stage (Leathwick et al., 2019).

For all simulations described here, it was assumed that resistance is the result of a single mutation in one gene, which is represented by three genotypes, a homozygous susceptible (SS), a homozygous resistant (RR) and a heterozygote (RS) with an initial resistance (R) gene frequency of 10^{-4}. For simplicity, only one anthelmintic is utilized (representing ivermectin) for which the efficacy was assumed to be 99%, 50% and 5% against the SS, RS and RR genotypes, respectively, against both adult worms and luminal L4, with no efficacy against any of the mucosal stages. The efficacies against SS stages were based on data in Klei and Torbert (1980); Klei et al. (2001), and Lyons et al. (1980). For every simulation the output of interest was the time (in model years) until the efficacy fell below 90% for a period of at least 30 days. The calculation of efficacy was based on the R-gene frequency of the pasture L3 population as outlined elsewhere (Sauermann et al., unpublished data), where the number of L3 in each resistance genotype is used to calculate the proportion which would be killed with the anthelmintic.

The model consists of a single population of free-living parasites (on pasture) but allows for multiple parasitic worm populations. All simulations assumed a group of 10 horses with an age structure chosen to mimic the typical overdispersed distribution of strongyle egg count levels observed in equine populations; 1, 2, 4, 6, 10, 14, 15, 16, 18, and 20 years of age. Note that, in the model, age is used as a proxy for horse immune status, which influences a number of parameters (Leathwick et al., 2019) including egg count levels. Thus, the ten horses in each simulation represent a range in immune status, between that of a one year old to a mature, immune competent horse. This remained unchanged, assuming the age structure of the herd stayed consistent throughout the 40 year simulation period.

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) and Deutscher Wetterdienst (www.dwd.de). For each climate zone a dataset consisting of daily max-min temperatures (°C) and rainfall (mm) covering a period of 10 years was sourced, and used as input data for the model, repeated four times to allow for a total simulation period of 40 years.

2.2. Comparison of treatment strategies

The anthelmintic treatment strategies evaluated were divided into three categories;

1 A single anthelmintic treatment administered once a year in any month of the year in each of the four contrasting climatic zones.
2 Treatment frequencies of six, four and two times a year (spring/autumn or summer/winter treatments) in the same four climates.
3 Evaluation of strategies with three possible treatment occasions in a year (April, June, and August) as generally outlined in current guidelines for equine parasite control (Nielsen et al., 2016). In one scenario, all horses received a treatment in August, while treatments in April and June were applied selectively based on strongyle egg counts. Alternatively, all horses were treated in April and August with the June treatment being egg count based. These were compared to scenarios, where all horses received treatment once, twice or three times during the year. For the selective treatments, two strongyle egg count thresholds were evaluated; 300 and 600 EPG. These scenarios were evaluated in all four climates described above and the number of individual treatments administered under these scenarios is outlined in Table 1.

3. Results

3.1. A single treatment administered at different times of the year in different climate zones

All of the climatic regions evaluated demonstrated within year differences in the rate at which resistance developed depending on which month of the year the single treatment was administered (Fig. 1). However, the differences between months were more pronounced in cold, temperate, and hot/cold semi-arid climates than in the humid subtropical climate. In general, treatments administered during winter in the colder climates were less selective for resistance than those given at other times of year.

Table 1
The number of anthelmintic treatments administered in the simulations evaluating strategies consisting of a combination of strategic treatments applied to all horses, and selective treatments administered to horses exceeding a strongyle fecal egg count threshold. Two different treatment thresholds (300 and 600 strongyle eggs per gram) were evaluated for four different climatic data sets (North Dakota, Germany, Texas, and Florida).

	Treatment threshold[a]	Strategic treatments	Selective treatments	Total treatments
Strategic treatments for all horses				
April, June, and August	NA	1200	0	1200
April and August	NA	800	0	800
August	NA	400	0	400
Two strategic treatments (April, August) and one selective treatment (June)				
North Dakota	300	800	56	856
	600	800	0	800
Germany	300	800	62	862
	600	800	0	800
Texas	300	800	52	852
	600	800	0	800
Florida	300	800	70	870
	600	800	12	812
One strategic treatment (August) and two selective treatments (April, June)				
North Dakota	300	400	126	526
	600	400	10	410
Germany	300	400	143	543
	600	400	10	410
Texas	300	400	131	531
	600	400	6	406
Florida	300	400	176	576
	600	400	38	438

[a] Strongyle fecal egg count thresholds given in eggs per gram (EPG) applied for selective anthelmintic therapy.

3.2. Frequency of treatment

All simulations demonstrated an effect of treatment frequency on the rate of resistance development (Fig. 2), with more annual treatments leading to a faster development of resistance, regardless of the climate zone. Furthermore, when only two treatments were administered each year, the timing of these influenced the rate of resistance development, particularly in the colder climates. Spring and autumn treatments were generally more selective for resistance than treatments administered in summer and winter.

3.3. Recommended strategies including selective treatment

Selective therapy (*i.e.,* leaving a proportion of horses untreated) had minimal impact on delaying resistance development if the evaluated scenario included a treatment of all horses in April, whereas scenarios with a selective treatment in April had a substantially slower development of resistance in the colder climates. Generally, a treatment threshold of 600 EPG always slowed resistance development compared to a threshold of 300 EPG (Fig. 3). However, as with other treatment strategies there was an effect of climate, with differences between strategies being less pronounced in the subtropical climate and more pronounced in the cold and temperate climates (Fig. 3).

A comparison of mean pasture third stage larval (L3) counts for model years 1, 5, 10, 20 and 40 is presented for the evaluated scenarios in the four different climates in Fig. 4. In general, a reduction of L3 counts was observed during initial years, but counts increased as resistance developed in all scenarios and all four climates. We also noted a substantial difference between climates.

4. Discussion

The development of this model has allowed, for the first time, an

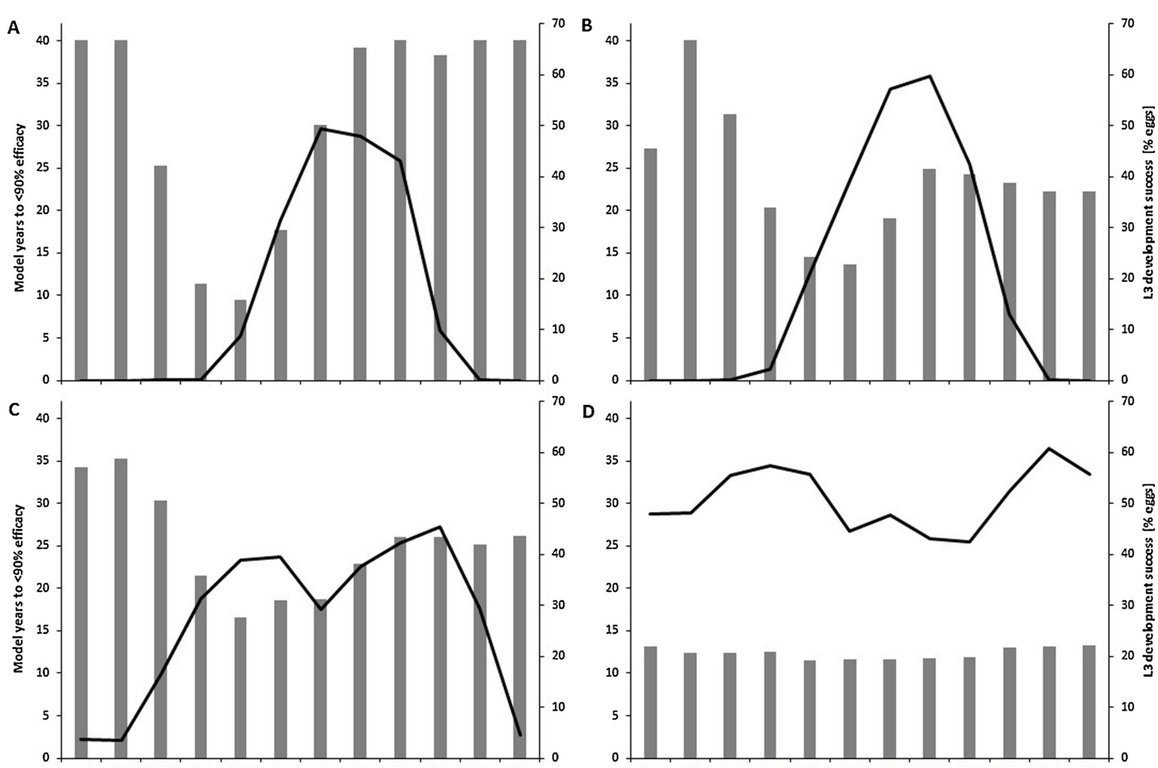

Fig. 1. Time (model years) for efficacy to decline below 90% (left Y-axes) when a single anthelmintic treatment (ivermectin) is administered to all horses once a year (bars) and the corresponding monthly percentage development of cyathostomin eggs to infective third stage larvae (L3) on pasture (black line, right Y-axes) replicated for weather data from A) North Dakota, USA B) Germany, C) Texas USA, and D) Florida, USA.

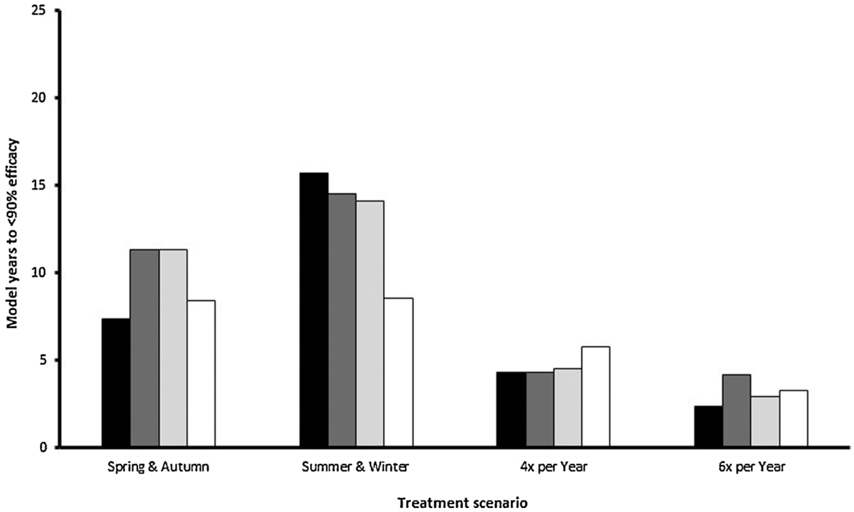

Fig. 2. Time (model years) for efficacy to decline below 90% in contrasting climates: North Dakota (black), Germany (dark grey), Texas (light grey, and Florida (white) using the following anthelmintic treatment scenarios; treatments in spring and autumn (March and September) each year, treatments in summer and winter (June and December) each year, 4 treatments each year and 6 treatments each year.

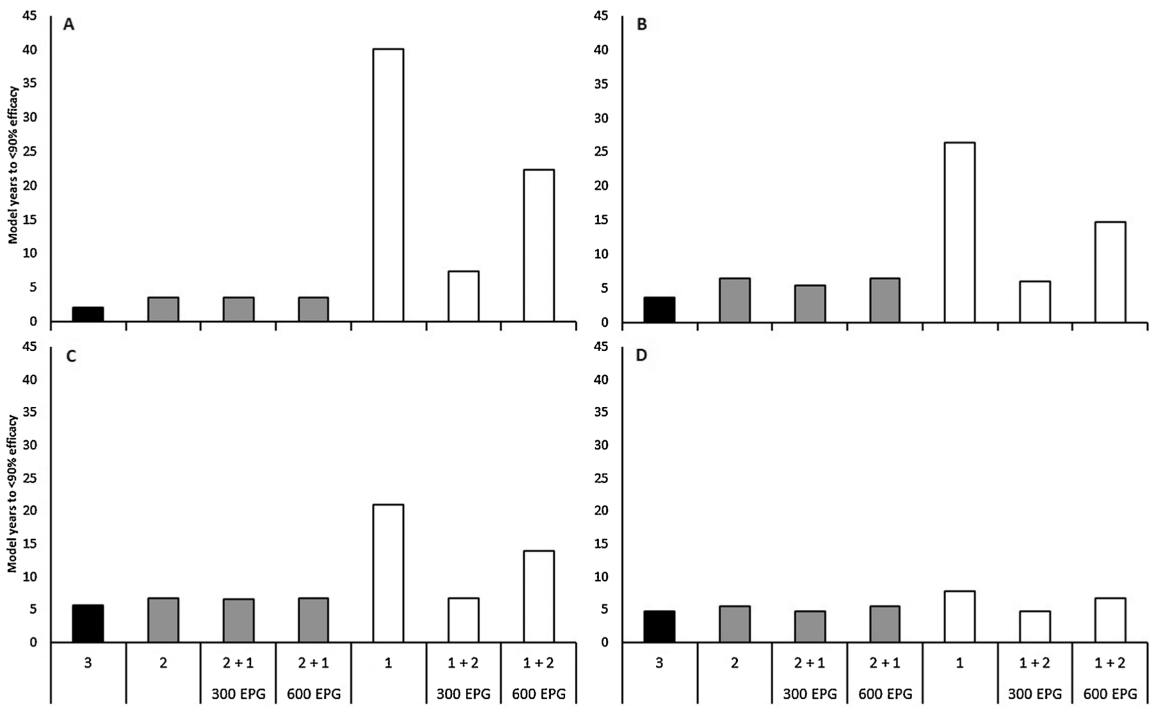

Fig. 3. Time (model years) for efficacy to decline below 90% when anthelmintic treatments are given to horses following recommended approaches in four different climates. The scenarios evaluated were divided into schedules with three (black columns), two (grey columns) or one treatment (white columns) administered to all horses. The following scenarios were modelled: Treating all horses in April, June, and August (3), treating all horses in April and August (2), Treating all horses in April and August with treating those exceeding a strongyle fecal egg count threshold in June (2 + 1) of either 300 or 600 eggs per gram of feces (EPG), treating all horses once per year in August (1), and treating all horses in August, while treating those exceeding a strongyle fecal egg count threshold of either 300 or 600 EPG in April and June (1 + 2). All scenarios were replicated for weather data from A) North Dakota, USA B) Germany, C) Texas USA, and D) Florida, USA.

investigation of the impact of different variables and treatment regimes on the development of anthelmintic resistance in cyathostomin parasites. Perhaps not surprisingly the results showed that treatment frequency was linked to the speed at which resistance developed. However, the results also indicated that reducing the number of annual treatments from six to four, while continuing to treat all horses, is likely to have only a minimal benefit in slowing resistance development. Currently, many horse owners are struggling to achieve even this modest level of reduction in the number of treatments administered (Robert et al., 2015; Becher et al., 2018; Nielsen et al., 2018). To achieve significant gains in slowing resistance development the model indicated that number of annual treatments as low as two may be necessary if all horses are still to be treated on every occasion.

At relatively low treatment frequencies, it appeared that timing of treatment (*i.e.,* month of the year) became an important consideration in certain climates. The model outputs demonstrated how resistance development can be significantly confounded by climate and seasonality patterns and the timing of a single treatment had a large impact on the development of resistance in three of the evaluated climates. In climates with a pronounced seasonality, such as North Dakota, Germany, and Texas, resistance was generally slower to develop when treatments were applied during winter and early spring (Fig. 1). These periods coincided with low rates of development of eggs to infective L3 on pasture, suggesting that worms surviving treatment at these times make a smaller contribution to subsequent pasture contamination. Furthermore, the one-year maximum life span of adult worms also

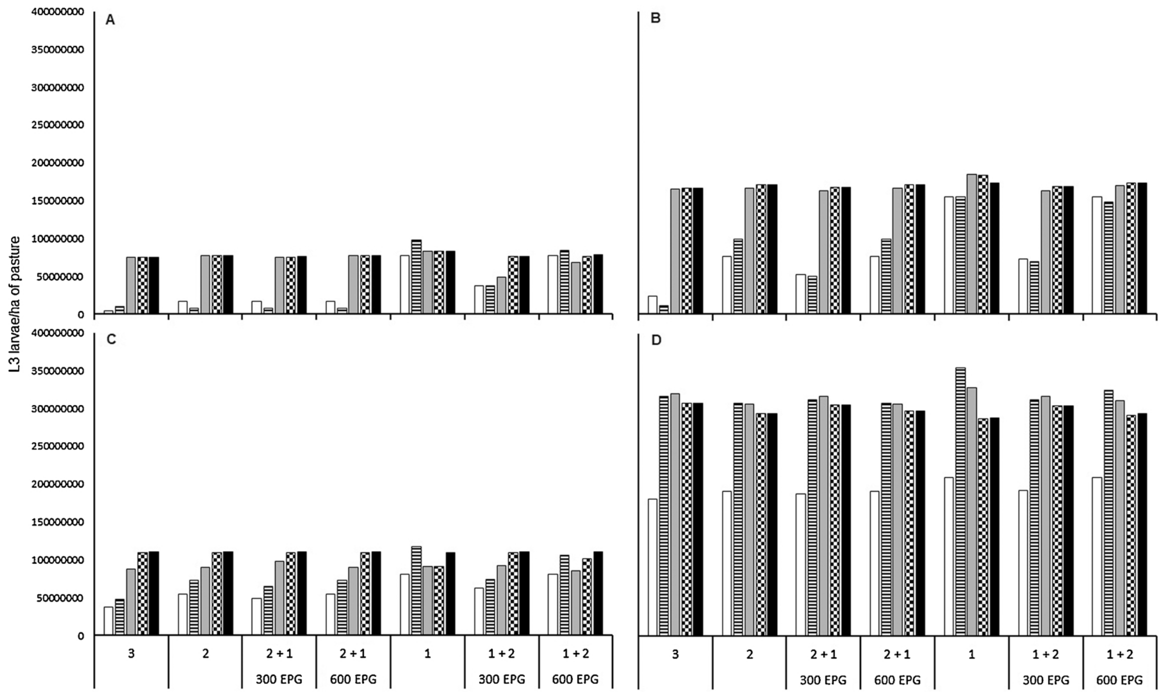

Fig. 4. Mean pasture counts per hectare of third stage cyathostomin larvae (L3) for Years 1 (white), 5 (striped), 10 (gray), 20 (checkerboard), and 40 (black) during the simulations. Data are presented for all evaluated scenarios of three (Apr-Jun-Aug), two (Apr-Aug) and one (Aug) yearly treatment occasions, with fecal egg count treatment thresholds of 0, 300, and 600 strongyle eggs per gram. All scenarios were replicated for weather data from A) North Dakota, USA B) Germany, C) Texas USA, and D) Florida, USA.

means that the resistant adult worms surviving treatment will be approaching their natural senescence in winter and so will soon be replaced by a newly developing adult worm population. This is likely to explain why resistance developed more quickly when treatments were administered during spring months in the two colder climates. Adult worms surviving treatments applied early in the year will be able to pass eggs onto pastures over the course of the entire grazing season. In contrast, in a subtropical climate offering year-round favourable conditions for cyathostomin transmission with limited or no seasonality, the time of the year had limited, if any, influence on the rate of resistance development. Again, this could be because of a less synchronized pattern with adult parasites establishing and being replaced over the course of the entire year. Regardless of the mechanism, the model outputs indicated that in some climatic regions, the timing of anthelmintic treatments may be worthy of consideration as a simple way for reducing the rate of resistance development.

A combination of one or two strategic treatments administered to all horses and one or two selective treatments administered to horses exceeding certain egg count levels had vastly different outcomes depending on whether all horses were treated in April or only selectively treated (Fig. 3). In the three climates with more pronounced seasonality (North Dakota, Germany, and Texas), resistance development was substantially delayed when selective treatments were carried out in spring (April), compared to the scenarios where all horses were treated in April. As discussed above, treatments administered early in the parasite transmission season will have a large impact on resistance development as surviving worms will live through the entire grazing season and contaminate the environment with resistance gene alleles. This suggests that selective therapy should be encouraged for spring treatments, whereas strategic treatments (administered to all horses) appear better suited for autumn treatments, where pasture contamination by the end of the grazing season is declining and adult worm burdens approaching the end of their life span. It is also worth noting that there were little or no benefits from practicing selective therapy, when the lower treatment threshold (300 EPG) was applied. This suggests that a treatment threshold in this range could be too low to be helpful. However, this needs further investigation before any conclusions can be drawn. Furthermore, it is also important to consider that several of the evaluated treatment scenarios provided limited apparent benefit in terms of reduction of pasture infectivity, and that there were substantial differences between climates (Fig. 4). This illustrates the complexity of equine parasite control; achieving a meaningful reduction in cyathostomin infection pressure without accelerating the development of anthelmintic resistance. Clearly, more work is needed in order to identify the balance between a necessary level of anthelmintic therapy protecting equine health and strategies aiming to reduce and delay anthelmintic resistance.

Three treatments a year administered to all horses led to resistance more quickly in the colder climates compared to Texas and Florida. This can also be explained by the less pronounced seasonality in those latter climates (Fig. 1), which means that parasite transmission happens year-round with ample opportunity for parasites to complete their life cycle without drug exposure. In contrast, three treatments had a larger impact in climates with shorter parasite transmission seasons. In other words, three treatments a year in North Dakota translated to higher treatment intensity than three treatments a year in a subtropical climate. It is also worth noting that selective therapy did not offer much benefit in those warmer climates for the scenarios evaluated. This seems to suggest that the lack of pronounced seasonality makes it challenging to devise a sustainable parasite control program and more work is needed to identify useful treatment strategies for this type of climate. Pasture management aiming at reducing infection pressure may be more important in these climates, and this should be evaluated in future studies.

In summary, this study has demonstrated that climate can be an important variable in resistance development, especially in climates with pronounced seasonality. The model suggested that treating all horses twice a year offered a marked reduction in the rate of resistance development compared to treating horses four or six times a year, but the timing of these (two) treatments in relation to climate was important. Finally, the model offered some insight into the possible value of currently recommended treatment regimens, where horses receive a combination of strategic treatments administered to all horses and selective treatments based on fecal egg count levels. However, results suggested that the effects are highly dependent on how treatments are carried out in the spring, and that these may differ largely between climates. Further studies – both in the field and with simulations - are needed to evaluate these findings further.

Conflict of interest statement

The authors declare no conflicts of interest.

Credit author statement

Dave Leathwick conceptualized the model, acquired the first funding and administered the project. Christian Sauermann organized the model and ran all simulations in R. Martin Nielsen supervised the project and prepared a first draft of the manuscript. All authors participated in reviewing and editing the paper.

Acknowledgements

The authors wish to thank Dr. Jay Donecker for initiating this project and Dr. Thomas Geurden for his encouragement and support. This work was principally funded by a grant from Zoetis with additional funding from AgResearch SSIF. The University of Kentucky, Department of Veterinary Science Clay Fellowship Fund supported Dr. Nielsen's sabbatical at AgResearch in 2018.

References

Becher, A.M., van Doorn, D.C., Pfister, K., Kaplan, R.M., Reist, M., Nielsen, M.K., 2018. Equine parasite control and the role of national legislation - a multinational questionnaire survey. Vet. Parasitol. 259, 6–12.
Duncan, J.L., Love, S., 1991. Preliminary observations on an alternative strategy for the control of horse strongyles. Equine Vet. J. 23, 226–228.
Geurden, T., van Doorn, D., Claerebout, E., Kooyman, F., De Keersmaecker, S., Vercruysse, J., Besognet, B., Vanimisetti, B., di Regalbono, A.F., Beraldo, P., Di Cesare, A., Traversa, D., 2014. Decreased strongyle egg re-appearance period after treatment with ivermectin and moxidectin in horses in Belgium, Italy and The Netherlands. Vet. Parasitol. 204, 291–296.
Gomez, H.H., Georgi, J.R., 1991. Equine helminth infections: control by selective chemotherapy. Equine Vet. J. 23, 198–200.
Herd, R.P., 1990. The changing world of worms – the rise of the cyathostomes and the decline of Strongylus vulgaris. Com. Cont. Educ. Pract. Vet. 12, 732–736.
Kaplan, R.M., Nielsen, M.K., 2010. An evidence-based approach to equine parasite control: it ain't the 60s anymore. Equine Vet. Educ. 22, 306–316.
Klei, T.R., Torbert, B.J., 1980. Efficacy of ivermectin (22,23-Dihydroavermectin-B_1) against gastrointestinal parasites in ponies. Am. J. Vet. Res. 41, 1747–1750.
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., Donecker, J.M., Nielsen, M.K., 2015. A model for the dynamics of the free-living stages of equine cyathostomins. Vet. Parasitol. 209, 210–220.
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.
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. 42, 2069–2072.
Lyons, E.T., Tolliver, S.C., Ionita, M., Lewellen, A., Collins, S.S., 2008. Field studies indicating reduced activity of ivermectin on small strongyles in horses on a farm in Central Kentucky. Parasitol. Res. 103, 209–215.
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.
Lyons, E.T., Tolliver, S.C., Collins, S.S., Ionita, M., Kuzmina, T.A., Rossano, M., 2011. Field tests demonstrating reduced activity of ivermectin and moxidectin against small strongyles in horses on 14 farms in Central Kentucky in 2007–2009. Parasitol. Res. 108, 355–360.
Menne, M.J., Williams, C.N.Jr., Vose, R.S., 2015. United States Historical Climatology Network Daily Temperature, Precipitation, and Snow Data. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
Nielsen, M.K., Mittel, L., Grice, A., Erskine, M., Graves, E., Vaala, W., Tully, R.C., French, D.D., Bowman, R., Kaplan, R.M., 2016. AAEP Parasite Control Guidelines. American Association of Equine Practitioners Online at: www.aaep.org.
Nielsen, M.K., Branan, M.A., Wiedenheft, A.M., Digianantonio, R., Garber, L.P., Kopral, C.A., Phillippi-Taylor, A.M., Traub-Dargatz, J.L., 2018. Parasite control strategies used by equine owners in the United States: a national survey. Vet. Parasitol. 250, 45–51.
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.
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., 1999. Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? Vet. Parasitol. 85, 189–204.
Tzelos, T., Barbeito, J.S., Nielsen, M.K., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2017. Strongyle egg reappearance period after moxidectin treatment and its relationship with management factors in UK equine populations. Vet. Parasitol. 237, 70–76.