

Strongyle Egg Reappearance Periods following Moxidectin Treatment in Horses in Southeast England

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Abstract

Background: A reduction in the Egg Reappearance Period (ERP) has been suggested to be an early indication of emerging anthelmintic resistance in strongyles. **Objective:** To measure the strongyle ERP following moxidectin treatment of horses in the southeast of England. **Study Design:** Prospective study. **Methods:** Horses with a faecal egg count (FEC) of > 400 strongyle eggs per gram (EPG) in a routine screening sample were enrolled into the study. Moxidectin (400 mcg/kg) was administered per os and FEC tests repeated every 2 weeks for 16 weeks. **Results:** Forty-eight horses completed the study. The mean EPG prior to treatment was 1047 (range 375 – 2137 EPG). In all but two horses, FEC was 0 EPG 2 weeks after moxidectin administration. In the remaining two, the FECs were 12.5 EPG (97.8-98.3% reductions compared to pre-treatment FEC). At 4 weeks post-treatment, 6 horses had positive FECs (96.6-99.2% reductions). At 6 weeks, 11 horses had positive FECs (83.8% reduction in one horse; >90% reduction in 10). At 8 weeks, 21 horses exhibited positive FECs (<90% reduction in 2). At 10 weeks, 27 horses had positive FECs (<90% in 6). At 12 weeks, 31 horses had positive FECs (<90% reduction in 11). At 14 weeks, 34 horses had positive FECs (<90% reduction in 13). At 16 weeks, 38 horses had positive FECs (<90% reduction in 17). **Limitations:** Weights of some horses were estimated using weigh tapes rather than a weighbridge. Dosing of the horses with moxidectin was carried out by owners. **Conclusions:** The results indicated acceptable efficacy of moxidectin at 14 days after treatment; however, the ERP pattern measured across the group suggest that this anthelmintic has a considerably shorter suppressive effect on strongyle egg shedding than measured when it was first introduced (>13 weeks and up to 24 weeks).

Introduction

Grazing horses are constantly exposed to parasites, with the cyathostomins (“small redworms”) (Strongylida: Cyathostominae) being the commonest. These parasitic nematodes encompass >40 species in 14 genera, but coinfections with 15–25 species are most common (Bellaw and Nielsen 2020). Current recommendations for cyathostomin control are aimed at reducing the risk of pasture contamination and minimising the chances of disease caused by the parasites (Matthews 2000 and 2011) using strategic or targeted treatments based on faecal egg count (FEC) analysis, and assessment of the efficacy of routinely used anthelmintics (Saeed *et al* 2019). Three classes of anthelmintic are licensed for the treatment of cyathostomin infections in the UK: the benzimidazoles (fenbendazole), the tetrahydropyrimidines (pyrantel salts) and the macrocyclic lactones (ivermectin and moxidectin). Resistance among the cyathostomins to the benzimidazole and tetrahydropyrimidine drugs is now widespread across the world (Kaplan *et al* 2004; Lester *et al* 2013; Matthews 2014; Peregrine *et al* 2014), and there is recent evidence to suggest growing resistance to the macrocyclic lactones (Relf *et al* , 2014; Nielsen *et al* 2018; Nielsen *et al* 2020; Floreset *et al* 2020; Abbas *et al* 2021; Nielsen *et al* 2022a), including in the UK (Bull *et al* 2023). However, reports suggest that in most regions, the macrocyclic lactones currently have considerably higher observed anthelmintic efficacy against strongyle infections than the other two drug classes (Nielsen *et al* 2018). Only two anthelmintics (fenbendazole administered daily for

five consecutive days and moxidectin as a single dose) are licensed for the treatment of cyathostomin mucosal larval stages in the UK. In view of the widespread resistance to the benzimidazole and tetrahydropyrimidine drugs, coupled with the efficacy profiles of the different anthelmintic compounds against mucosal larval stages, routine treatment of all grazing horses with moxidectin (often combined with praziquantel to treat tapeworms) in the autumn/early winter is commonly practised in the UK, but this is likely adding to the selection pressure on the cyathostomins for developing resistance (Rendle 2017).

The egg reappearance period (ERP) is defined as the time between the administration of an effective anthelmintic and the recommencement of shedding of parasite eggs in faeces (Nielsen *et al* 2019). The determination of ERP following anthelmintic treatment has been suggested as a useful indicator for the early development of anthelmintic resistance in cyathostomins. A number of studies have been published that have assessed ERP following moxidectin treatment (reviewed by Macdonald *et al* 2023) and, in the past two decades, several of these have reported reduced ERP following treatment with moxidectin, suggesting that resistance to this compound is emerging. In view of the widespread routine use of moxidectin in competition and pleasure horses across all regions of the UK, information about how effective moxidectin is against cyathostomin populations in different regions of the country will be valuable in order to inform local guidelines for parasite control. The aims of this study were to evaluate the ERP following moxidectin in competition and pleasure horses in the southeast of the UK.

Materials and Methods

All routine FECs performed at Bell Equine Veterinary Clinic in southeast England during a 3-month period (August to November 2017) were assessed and owners of horses with FEC greater or equal to 400 strongyle eggs per gram (EPG) were invited to participate in the study. The FECs were processed using a modified McMaster technique with a sensitivity 12.5 EPG. Following enrolment of a horse into the study, a repeat FEC was performed within two weeks of the first, and the mean of the two values for that horse was recorded. The horses' weight were measured using either a weigh tape only (n=10), a weighbridge only (n=6), or both a weigh tape and a weighbridge (n=32), and oral moxidectin was administered by the owners at a dose rate of 400 mcg/kg *per os* immediately (within 24 hours) following sampling for the second FEC. Faecal egg counts were subsequently performed every 2 weeks for 16 weeks. No further doses of anthelmintics were administered during this period.

Results

A total of 52 horses fulfilled the initial inclusion criteria. Four were excluded due to incomplete FEC data, leaving 48 horses that completed the study. These 48 horses were kept at 17 different premises, including 9 horses at private yards, 35 at livery yards and 4 at racing yards. Age was recorded in 46 horses (mean age 11.8 years; range 1 – 26 years). Breeds were recorded in 44 horses (15 ponies, 8 Thoroughbreds, 8 warmbloods, 8 sports horses, 4 cobs and 1 draught horse). There were 17 mares and 31 geldings. The mean number of horses grazing on the same pasture as the faecal sampled horses was 3 (range 1 – 11) (stocking rate was not recorded). Pasture hygiene (retrieval of faeces from the pasture, “poo-picking”) was routinely performed on 29 of the premises (including 15 where the owners reported “regularly doing this daily”, 8 where this was done “2-3 times a week”, one where this was done “weekly”, and 7 where this was done “irregularly in combination with harrowing”). The horses grazed, on average, 15 hours per day (range 2 – 24 hours) during the study period (August 2017-March 2018).

The mean FEC prior to treatment was 1047 EPG (range 375 – 2137). The numbers of horses with positive FECs and numbers of horses with FEC reduction <90% compared to the pre-moxidectin treatment FEC at the different time points are shown in Figure 1. Two horses had a positive FEC 2 weeks after dosing with moxidectin; both had a FEC of 12.5 EPG, which represented reductions of 97.8% and 98.3% compared to the mean pre-treatment FEC. All other horses had a 0 FEC at 2 weeks. At 4 weeks post-treatment, 6 horses had positive FECs representing percentage reductions compared to the pre-treatment FEC (i.e., faecal egg count reduction (FECR)) of between 96.6% and 99.2%. At 6 weeks post-treatment, 11 horses had positive FECs; one of these had a FECR of 83.8%, whilst the others were all >90%. At 8 weeks, 21 horses had

positive FECs; 2 of these had a FECR of <90% (73.2% and 83.8%), whilst the others were all >90%. At 10 weeks, 27 horses had positive FECs; 6 of these had a FECR of <90% (50.0% to 89.6%), whilst the others demonstrated FECRs >90%. At 12 weeks, 31 horses had positive FECs; 11 of these had a FECR of <90% (range 55.7% to 89.6%), whilst the others were all >90%. At 14 weeks, 34 horses had positive FECs; 13 of these horses had a FECR of <90% (range 50.0% to 89.6%), whilst the others were all >90%. At 16 weeks, 38 horses had positive FECs; 17 of these horses had a FECR of <90% (range 50.0% to 89.6%), whilst the others were all >90%.

Discussion

None of the 48 horses in this study had a faecal egg count reduction test (FECRT) result at 2 weeks after moxidectin that indicated resistance to this anthelmintic. Resistance to moxidectin has, however, been recently identified in yearlings on a Thoroughbred stud farm in England (Bull *et al* 2023) and resistance to ivermectin has been reported on a Thoroughbred farm in the USA in animals imported from Ireland (Nielsean *et al* 2020). The results of the latter study serve to illustrate that the global movement of horses has the potential to spread macrocyclic lactone-resistant parasite isolates around the world. As a result, it is important that the equine industry routinely monitors anthelmintic efficacy, so the occurrence of macrocyclic lactone resistant cyathostomins can be detected and appropriate interventions implemented as early as possible (Nielsen *et al* 2020). The current study was undertaken with this purpose in mind, to assess whether there is any evidence of moxidectin resistance in leisure and competition horses in southeast England and to monitor the ERP following moxidectin treatment. Shortened ERPs have been regarded as an early indicator of anthelmintic resistance (Sangster 1999). Original studies reported the ERP of moxidectin to be >13 weeks and up to 24 weeks (Jacobs *et al* 1995). Although two horses in the current study had positive FEC results at two weeks after moxidectin treatment, in both cases, the percentage FEC reduction compared to the pre-treatment FEC results was in the 95-100% range, which is considered to indicate effective efficacy (Nielsen *et al* 2018). However, from 6 weeks onwards, an increasing proportion of the horses had FECRT results indicating suspect ineffective (85-95%) or ineffective (<85%) efficacy (Nielsen *et al* 2018). These results are compatible with the current definitions of reduced ERP, which might herald the development of resistance in cyathostomin populations.

Several different definitions of ERP have been described including: 1. Detection of the first FEC-positive horse following deworming (Lyons *et al* 2008); 2. Defining a fixed threshold FEC, which 50% of horses tested (Demeulenaere *et al* 1997) or the group mean (Boersema *et al* 1996) exceeds; and 3. Calculating the percent FECR and defining a mean efficacy threshold for estimates to fall below (Samson-Himmelstjerna *et al* 2007). The first of these methods was used in the present study; two of the 48 horses (4%) had positive strongyle FEC results at 2 weeks after treatment. In addition, reported here are the number of individual horses at each time point when the percentage FECRT result fell below the 95% range, which is considered to indicate effective efficacy (Nielsen *et al* 2018). Also reported are the numbers of horses with FECR <90% at the different timepoints following moxidectin treatment; the number below this threshold showed a steady increase from 6 weeks (one horse, 2%), to 2 horses (4%) at 8 weeks, to 6 horses (12%) at 10 weeks, to 11 horses (23%) at 12 weeks, to 13 horses (27%) at 14 weeks and 17 horses (35%) at 16 weeks (Figure 1). However, it should be noted that the results of FECRTs are reported for individual horses in this study, whereas it is generally recommended that FECRTs be performed in groups of horses (>6) and mean results used to determine whether or not potential resistance present. In the current study, individual horses with high FEC results were pre-selected to monitor FECR and FEC data, and the results should be interpreted in light of this.

No attempt was made here to differentiate between different species of strongyle eggs contributing to the positive FEC results. Therefore, it was not possible to be certain that there were no *Strongylus vulgaris* eggs counted in the samples. However, it is more than likely that 100% of the observed strongyle eggs were of cyathostomin species. In recent studies of faecal egg shedding from horses in the UK, *S. vulgaris* was not observed in coprocultures, nor using a *S. vulgaris* -specific end-point PCR; larval identification analysis showed that on all premises, parasites recovered before and after anthelmintic treatment were cyathostomins

(Tzelos *et al* 2017). Moreover, there have been no reports of resistance in *S. vulgaris* to moxidectin.

Identification of a reduction of the ERP following anthelmintic treatment has been considered as an early indicator of emerging anthelmintic resistance (Sangster, 1999). However, it is unclear whether the observed reducing ERP profiles of macrocyclic lactone anthelmintics are due solely to the acquisition of anthelmintic resistance, or to other factors related to individual horses, cyathostomin species, and/or the environmental conditions (Nielsen *et al* 2022; Macdonald *et al* 2023). There have been a limited number of previous studies of the ERP following moxidectin treatment performed in the UK horse population. Relf *et al* (2014) assessed anthelmintic efficacy in 161 horses on 7 stud farms in England. No resistance to moxidectin (as determined by FECRT at 14 days) was recorded in this study. Strongyle ERP data were collected from three farms and two methods for calculating ERP applied; the strongyle ERP recorded ranged from 4–9 weeks. Similarly, Daniels and Proudman (2016) analysed ERP following moxidectin in 95 pleasure horses from across the UK and the shortest ERP recorded in this study was 5 weeks, and in 5 of 16 premises where moxidectin was used, ERP (measured as >10% of day 0 FEC and FECR <90%) was 12–13 weeks. However, the horses selected for inclusion in the Daniels and Proudman (2016) study had previous signs of early egg re-appearance, thus biasing the sample towards horses possibly harbouring strongyles with reduced susceptibility to moxidectin. Tzelos *et al* (2017) reported the ERP after moxidectin in 261 horses on 8 yards distributed across England, Scotland and Wales. The strongyle ERP in the latter study ranged from 6 weeks to >12 weeks depending on the calculation method applied. The results of the current study are broadly in agreement with these previous studies from the UK. Future work to repeat monitoring of ERPs following moxidectin will be undertaken to establish if further reductions in ERP are arising.

It should be noted that cyathostomin prepatent periods and ERPs may have been affected by the age of the equines studied. In a group of 4–5-year-old ponies, Smith (1976) determined that strongyle egg shedding resumed at 12–15 weeks post-inoculation. However, when the author repeated the study protocol with the same ponies six years later, the prepatent period/ERP was observed to be 17–18 weeks (Smith, 1978), although factors other than or in addition to age may have been involved in the different observations between these studies. Thus, it has been recommended that ERP data should include the age range of the equines studied (Nielsen *et al* 2022b). In the current study, the mean age of the 46 horses where this was recorded was 11.8 years, with only 5 horses less than 5 years old, and so most individuals examined were in the adult category.

There are a number of potential limitations of this study. Firstly, the weights of 10/48 horses were estimated using weigh tapes rather than accurately determined by a weigh bridge. In addition, dosing of the horses with moxidectin was carried out by owners and not observed by the researchers, so it is possible that not all horses received the true calculated dose of moxidectin. Underdosing of moxidectin could potentially have resulted in reduced efficacy of the drug in some instances; however, the absence of a positive FECRT result at two weeks in any horse suggests that effective doses were likely to have been administered. Despite these limitations, this study clearly demonstrates that the ERP following moxidectin treatment in this group of horses is considerably shorter than that reported when this anthelmintic was first licenced. The results therefore warrant caution in regards to the frequency of use of this anthelmintic and indicate that more evidence-based approaches, using diagnostics, should be applied to avoid drug overuse. The results also provide up-to-date information that can be used to inform the frequency of FEC testing that should be applied in UK horse populations in order that horses shedding high levels of strongyle eggs onto pasture be detected promptly so that they can be appropriately treated to reduce contamination onto grazing.

References

Abbas, G., Ghafar, A., Hurley, J., Bauquier, J., Beasley, A., Wilkes, E.J.A., Jacobson, C., El-Hage, C., Cudmore, L., Carrigan, P., Tennent-Brown, B., Gauci, C.G., Nielsen, M. K., Hughes, K.J., Beveridge, I., Jabbar, A. (2021) Cyathostomin resistance to moxidectin and combinations of anthelmintics in Australian horses. *Parasites Vectors* **14** 597

Bellaw, J.L. and Nielsen, M.K. (2020) Meta-analysis of cyathostomin species-specific prevalence and relative

abundance in domestic horses from 1975–2020: emphasis on geographical region and specimen collection method. *Parasites Vectors* **13** 509

Boersema, J.H., Eysker, M., Maas, J., and van der Aar, W.M. van (1996) Comparison of the reappearance of strongyle eggs in foals, yearlings, and adult horses after treatment with ivermectin or pyrantel *Vet.Q.* **18** 7-9

Bull, K.E., Allen, K.J., Hodgkinson, J.E. and Peachey, L.E. (2023) The first report of macrocyclic lactone resistant cyathostomins in the UK. *Int.J.Parasitol.Drugs Drug Resist.* **21** 125-130

Daniels, S.P. and Proudman, C.J. (2016) Shortened egg reappearance after ivermectin or moxidectin use in horses in the UK. *Vet.J.* **218** 36–39

Demeulenaere, D., Vercruysse, J., Dorny, P. and Claerebout, E. (1997) Comparative studies of ivermectin and moxidectin in the control of naturally acquired cyathostome infections in horses. *Vet.Rec.* **15** 383–386

Flores, A.G., Osmari, V., Ramos, F., Marques, C.B., Ramos, D.J., Botton, A.S., Vogel, F.S. F. and Sangioni, L.A. (2020) Multiple resistance in equine cyathostomins: a case study from military establishments in Rio Grande do Sul, Brazil. *Braz.J.Vet.Parasitol.* **29**, e003820

Jacobs, D.E., Hutchinson, M.J., Parker, L. and Gibbons, L.M. (1995) Equine cyathostome infection: Suppression of faecal egg output with moxidectin. *Vet.Rec.* **137** 545

Kaplan, R.M., Klei, T.R., Lyons, E.T., Lester, G., Courtney, C.H., French, D.D., Tolliver, S. C., Vidyashankar, A.N., Zhao, Y. (2004) Prevalence of anthelmintic resistant cyathostomes on horse farms. *J.Am. Vet.Med.Assoc.* **225** 903–910

Lester, H.E., Spanton, J., Stratford, C.H., Bartley, D.J., Morgan, E.R., Hodgkinson, J.E., Coumbe, K., Mair, T., Swan, B., Lemon, G., Cookson, R. and Matthews, J.B. (2013) Anthelmintic efficacy against cyathostomins in horses in Southern England. *Vet.Parasitol.* **197** 189-196

Lyons, E.T., Tolliver, S.C., Ionita, M., Lewellen, A. and 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

Macdonald, S.L., Abbas, G., Ghafar, A., Gauci, C.G., Bauquier, J., El-Hage, C., Tennent-Brown, B., Wilkes, E.J.A., Beasley, A., Jacobson, C., Cudmore, L., Carrigan, P., Huley, J., Beveridge, I., Hughes, K.J., Nielsen, M.K. and Jabbar, A. (2023) Egg reappearance periods of anthelmintics against equine cyathostomins: The state of play revisited. *Int.J.Parasitol.Drugs Drug Resist.* **21** 28-39

Matthews, J.B. (2014) Anthelmintic resistance in equine nematodes. *Int.J.Parasitol.Drugs Drug Resist.* **4** 310–315

Matthews, J.B. (2000) An update on cyathostomins: Anthelmintic resistance and worm control *Equine Vet.Educ.* **20** 552-560

Matthews, J.B. (2011) Facing the threat of equine parasitic disease. *Equine Vet.J.* **43** 126-132

Nielsen, M.K., Banahan, M. and Kaplan, R.M. (2020) Importation of macrocyclic lactone resistant cyathostomins on a US thoroughbred farm. *Int.J.Parasitol. Drugs Drug Resist.* **14** 99–104

Nielsen, M.K., Branan, M.A., Wiedenheft, A.M., Digianantonio, R., Scare, J.A., Bellaw, J.L., Garber, L.P., Koprak, C.A., Phillippi-Taylor, A.M. and Traub-Dargatz, J.L. (2018) Anthelmintic efficacy against equine strongyles in the United States. *Vet.Parasitol.* **259** 53-60

Nielsen, M.K., Mittel, L., Grice, A., Erskine, M., Graves, E., Vaala, W., Tully, R.C., French, D.D., Bowman, R. and Kaplan, R.M. (2019) AAEP Parasite Control Guidelines. American Association of Equine Practitioners, Lexington. <https://aaep.org/document/internal-parasite-control-guidelines>

Nielsen, M.K., Littman, B.A., Orzech, S.W. and Ripley, N.E. (2022a) Equine strongylids: ivermectin efficacy and fecal egg shedding patterns. *Parasitol.Res.* **121** 1691-1697

- Nielsen, M.K., Samson-Himmelstjerna, G von, Kuzmina, T.A., Doorn, D.C.K. van, Meana, A, Rehbein, S., Elliott, T. and Reinemeyer, C.R. (2022b) World association for the advancement of veterinary parasitology (WAAVP): Third edition of guideline for evaluating the efficacy of equine anthelmintics. *Vet.Parasitol.* **303** 109676
- Nielsen, M.K., Steuer, A.E., Anderson, H.P., Gavriluc, S., Carpenter, A.B., Redman, E.M., Gilleard, J.S., Reinemeyer, C.R. and Poissant, J. (2022c) Shortened egg reappearance periods of equine cyathostomins following ivermectin or moxidectin treatment: morphological and molecular investigation of efficacy and species composition.*Int.J.Parasitol.* **52** 787-798
- Peregrine, A.S., Molento, M.B., Kaplan, R.M. and Nielsen, M.K. (2014) Anthelmintic resistance in important parasites of horses: does it really matter? *Vet.Parasitol.* **201** 1–8
- Relf, V.E., Lester, H.E., Morgan, E.R., Hodgkinson, J.E. and Matthews, J.B. (2014) Anthelmintic efficacy on UK Thoroughbred stud farms.*Int.J.Parasitol.* **44** 507–514
- Rendle, D. (2017) Autumn de-worming in adult horses. *UK-Vet Equine* **1** 6-10
- Samson-Himmelstjerna, G. von, Fritzen, B., Demeler, J., Schurmann, S., Rohn, K., Schnieder, T. and C. Epe, C. (2007) Cases of reduced cyathostomin egg-reappearance period and failure of Parascaris equorum egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. *Vet.Parasitol.***144** 74-80
- Sangster, N. (1999) Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? *Vet.Parasitol.* **85** 189–204
- Saeed, M.A., Beveridge, I., Abbas, G., Beasley, A., Bauquier, J., Wilkes, E., Jacobson, C., Hughes, K.J., El-Hage, C., O’Handley, R., Hurley, J., Cudmore, L., Carrigan, P., Walter, L., Tennent-Brown, B., Nielsen, M.K. and Jabbar, A., (2019) Systematic review of gastrointestinal nematodes of horses from Australia. *Parasites Vectors* **12** 188
- Smith, H.J. (1976) Strongyle infections in ponies. II. Reinfection of treated animals. *Can.J.Comp.Med.* **40** 334–340
- Smith, H.J. (1978) Experimental *Trichonema* infections in mature ponies. *Vet.Parasitol.* **4** 265–273
- Tzelos, T., Barbeito, J.S.G., Nielsen, M.K., Morgan, E.R., Hodgkinson, J.E. and 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