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The first report of macrocyclic lactone resistant cyathostomins in the UK

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ABSTRACT

In recent years, resistance to the benzimidazole (BZ) and tetrahydropyrimidine (PYR) anthelmintics in global cyathostomin populations, has led to reliance on the macrocyclic lactone drugs (ML-of which ivermectin and moxidectin are licensed in horses) to control these parasites. Recently, the first confirmed case of resistance to both ivermectin (IVM) and moxidectin (MOX) was reported in the USA in yearlings imported from Ireland. This suggests that ML resistance in cyathostomins has emerged, and raises the possibility that regular movement of horses may result in rapid spread of ML resistant cyathostomins. Resistance may go undetected due to a lack of surveillance for ML efficacy. Here, we report anthelmintic efficacies in cyathostomins infecting UK Thoroughbreds on four studs. Faecal egg count reduction tests (FECRT) were performed to define resistance (resistance = FECR <95% lower credible interval (LCI) < 90%). Stud A yearlings had FECRs of 36.4–78.6% (CI:15.7–86.3) after three IVM treatments, 72.6% (CI: 50.8–85.2) after MOX, and 80.8% (CI: 61.9–90.0) after PYR. Mares on stud A had a FECR of 97.8% (CI: 93.3–99.9) and 98% (95.1–99.4) after IVM and MOX treatment, respectively. Resistance to MLs was not found in yearlings or mares on studs B, C or D with FECR after MOX OR IVM treatment ranging from 99.8 to 99.9% (95.4–100); although yearlings on studs B, C and D all had an egg reappearance period (ERP) of six weeks for MOX and stud C had a four-week ERP for IVM. This study describes the first confirmed case of resistance to both licensed ML drugs on a UK Thoroughbred stud and highlights the urgent need for a) increased awareness of the threat of ML resistant parasites infecting horses, and b) extensive surveillance of ML efficacy against cyathostomin populations in the UK, to gauge the extent of the problem.

1. Introduction

Cyathostomins are the most prevalent species of endoparasite infecting equids worldwide (Nielsen, 2012); and can be associated with significant pathology in youngstock and immune compromised horses (Love et al., 1999; Chapman et al., 2003; Kornaś et al., 2010; Relf et al., 2013; Nielsen et al., 2020b). It has been reported that some horses exposed to high infection levels are at risk of a severe protein losing enteropathy known as larval cyathostominosis, characterized by protein losing enteropathy resulting from mass emergence of larvae from the mucosa into the intestinal lumen. This condition tends to have a seasonal occurrence, presenting more often in spring and summer and leads to diarrhea, oedema, weight loss and abdominal pain, with a mortality rate reported of up to 50% (Hillyer and Mair, 1997; Love et al., 1999; Lyons et al., 2000). In temperate regions, such as the UK, it has been common practice to routinely apply larvicidal anthelmintics in the late autumn/winter, to remove a proportion of early third larval stage (EL3)/L4 larvae from the mucosa prior to the high-risk period for mass emergence (Xiao et al., 1994).

Currently only three classes of anthelmintic are available for use against cyathostomins, benzimidazoles (BZs, e.g fenbendazole, FBZ), tetrahydropyrimidines (e.g pyrantel, PYR) and macrocyclic lactones (MLs, e.g ivermectin (IVM) and moxdectin (MOX)); with MOX (at 0.4 mg/kg single dose) and FBZ (at 7.5 mg/kg for five days) the only drugs labelled for larvicidal efficacy.

In the UK, due to development of drug resistance in cyathostomin populations FBZ efficacy is reported to be universally low, following studies in England, n = 101 horses from 12 yards (Lester et al., 2013) and Scotland, n = 55 horses from 7 yards, (Stratford et al., 2014). These studies showed that, at the time, several cyathostomin populations remained susceptible to PYR, for example Lester et al. (2013) found that just two of 12 yards had evidence of PYR resistance using faecal egg count reduction test (FECRT) and Stratford et al. (2014) reported an overall efficacy of 90.4–99.6% in Scottish yards. This was consistent with earlier studies that showed only four out of 22 yards in the UK to have PYR resistance (Traversa et al., 2009). However, many studies

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suggest that PYR resistance is now prevalent, both in the UK and worldwide (Ihler, 1995; Tarigo-Martinie et al., 2001; Kaplan et al., 2004; Meier and Hertzberg, 2005; Lind et al., 2007; Molento et al., 2008; Milillo et al., 2009; Traversa et al., 2009; Nielsen et al., 2013; Geurden et al., 2014; Relf et al., 2014; Stratford et al., 2014; Fischer et al., 2015; Dauparaitė et al., 2021; Zanet et al., 2021).

With poor confidence in FBZ and PYR, the MLs, have become the mainstay of cyathostomin treatment within the UK and globally. The efficacy of MOX against encysted larvae has been reported to be 63.6% and 73.8% against EL3, and 85.2% and 75.6% against LL3/L4 mucosal cyathostomins, respectively (Reinemeyer et al., 2015; Bellaw et al., 2018). MOX is commonly used to target encysted larvae, with the aim of reducing the risk of larval cyathostominosis, particularly in areas where larval cyathostominosis is a concern, such as the UK. To date there have been very few reports of confirmed ML resistance in cyathostomin populations. The existing reports used low numbers of animals and FEC reductions were marginal and appeared to be driven by only one animal, with no repeat testing performed to confirm the result (Molento et al., 2008; Traversa et al., 2009; Canever et al., 2013; Relf et al., 2014), raising the question of whether these data represent true ML resistance. However, it is clear that there have been an increasing number of studies reporting the egg reappearance period (ERP) to be shorter after treatment with MLs than originally reported (Lyons et al., 2010; Rossano et al., 2010; Tzelos et al., 2017; Molena et al., 2018), implying that the efficacy of MLs has been reducing in recent years. Yet there remains an almost total reliance on MLs for cyathostomin control, and a lack of ongoing surveillance for emerging resistance.

Importantly, the first report of both IVM and MOX resistance in cyathostomins was described recently (Nielsen et al., 2020a). These ML-resistant parasites were detected on a stud in the USA in Thoroughbred (TB) yearlings that had recently been imported from Ireland. This report of resistance is of significant concern as it represents introduction of resistant parasites from animals imported from Europe, highlighting the risk of disseminating ML resistant parasites due to movement of horses around the world. There is no indication that novel anthelmintics are being developed for, or likely to be licensed for use in horses in the near future and a lack of widespread surveillance for ML resistance in cyathostomins raises the question of how extensive the problem of ML resistance is.

In this report, we explored the efficacy of MLs against cyathostomin populations infecting youngstock and mares on four TB studs in England, by means of FECRTs.

2. Materials and methods

2.1. Horses

Feacal samples were collected from horses on four TB studs in England. Between five and 55 individuals (yearlings or mares) were sampled within each treatment group, for all timepoints in 2021 and 2022. At the time of sampling Stud A had approximately 100 mares and 85 yearlings. Stud B had 14 yearlings and 16 mares. Stud C had approximately 25 yearlings and 30 mares and Stud D had 20 yearlings and 30 mares. Mares were only sampled on stud A and B as mares on studs C and D did not have high enough FECs to meet our inclusion criteria as per American Association of Equine Practitioners (AAEP) and World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Nielsen et al., 2013; Nielsen et., 2022).

2.2. Faecal egg count reduction test method

To determine FECR and strongyle ERP samples were collected the week before anthelmintic treatment and thereafter every seven days on stud A and every 14 days on stud B, C and D until the mean egg count of the group had risen to at least 10% of the original FEC value. Faecal samples were collected by observing the horse in the field or barn until defecation. Samples were collected from the ground with a gloved hand and put into individually labelled zip lock bags. Samples were stored at 4 °C and processed within five days of collection.

Mini-Flotac was used with saturated saline (specific gravity 1.18) to conduct the FEC, which has a sensitivity of 5 eggs per gram (EPG) (Cringoli et al., 2010, 2017). Each sample was counted twice and an average of the two counts recorded and used for analysis. Individuals with a FEC of at least 100 EPG before treatment were included in the first anthelmintic treatments for both mares and yearlings. This value was used as it was the lowest threshold for treatment amongst the four studs.

2.3. Treatments administered

Treatments that were followed during the course of this study are listed in Table 1. The decision of when to treat, with which anthelmintic and ensuring accurate dosage was solely at the discretion of the stud manager and veterinary surgeon. The following drugs were used on the studs: IVM and praziquantel (PRZ) combination (Aloquantel oral gel, 0.2 mg/kg IVM + 1.5 mg/kg PRZ, Virbac), IVM (Eqvalan Oral Paste, 0.2 mg/kg, Boehringer Ingelheim Animal Health UK Ltd), MOX (Equest Oral Gel, 0.4 mg/kg, Zoetis UK Limited), PYR (Alonate-P 19 mg/kg, Bimeda Animal Health Limited) and MOX and PRZ combination (Equest Pramox 0.4 mg/kg MOX + 2.5 mg/kg PYR, Zoetis UK Limited). Following initial testing using a generic product, the FECRT for IVM (Eqvalan Oral Paste, 0.2 mg/kg, Boehringer Ingelheim Animal Health UK Ltd) was repeated on stud A in yearlings to confirm IVM resistance. PYR treatment was also given to the yearlings on stud A during the study, and thus we performed a FECRT for PYR. Furthermore, on stud A, the FECRT was repeated in the next cohort of yearlings in the following year, to evaluate whether resistant cyathostomin populations were transmitted between cohorts in successive years.

2.4. Statistical analysis

The efficacies of each drug were calculated using the arithmetic mean (AM) EPG values pre and 14 days post-treatment, with the FECRT calculated using the formula: $(AM_{pre} - Am_{post})/AM_{pre} \times 100$.

The online web interface package eggCounts (http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/) was used to calculate the mean FECR for each of the treatment groups. This uses Bayesian hierarchical models and is recommended by the WAAVP guidelines (Torgerson et al., 2014; Wang et al., 2018; Nielsen et al., 2022). Resistance was defined in cases where the lower credible interval was below

Table 1
Treatments administered to yearling and mare groups on studs A-D.

Stud	Group (yearlings/ mares)	Treatment date	Drug	Product	Pasture or Stable
A	Y	Mar-21	IVM + PRZ	Aliquantel	S
A	Y	Apr-21	IVM	Eqvalan	P
A	Y	June-21	MOX	Equest	P
A	Y	Aug-21	PYR	Alonate-P	P
A	Y	Jan-22	IVM + PRZ	Aliquantel	S
A	M	Apr-21	IVM + PRZ	Aliquantel	S
A	M	July-21	MOX + PRZ	Equest Pramox	P
B	Y	May-21	MOX + PRZ	Equest Pramox	P
B	M	July-21	MOX + PRZ	Equest Pramox	P
C	Y	June-21	IVM	Eqvalan	P
C	Y	Mar-22	MOX + PRZ	Equest Pramox	P
D	Y	May-22	MOX	Equest	P

90% and the mean FECR of the group was below 95% (Coles et al., 1992). R studio was used to plot mean FEC at each of the timepoints sampled (R studio, 2020).

3. Results

Individual analysis of each treatment group was undertaken to determine the FECR (Table 2). In the yearling groups on stud A none of the anthelmintics tested showed a reduction of over 95% with a lower credible interval above 90%, suggesting resistance to IVM, MOX and PYR in the 2021 sampled yearlings. Only IVM was tested in the 2022 cohort of yearlings and again resistant cyathostomins were found infecting these youngstock (Table 1). Yearlings on stud A had FECRs ranging from 36.4 to 78.6% (CI:15.7–86.3) after treatment with IVM, 72.6% (CI: 50.8–85.2) after treatment with MOX, and 80.8% (CI: 61.9–90.0) after treatment with PYR. Yearlings on stud C had an ERP of four weeks for IVM (Fig. 1A) with FECR of 98% (95.4–99.2) (Fig. 1A). Studs B, C and D did not appear to have resistance to MOX based on the yearling groups tested, with FECR ranging from 99.8 to 99.9% (98.4–100) and all three studs had a strongyle ERP of six weeks for MOX (Fig. 2A).

In the mare groups on studs A and B, anthelmintic resistance was not confirmed for either IVM or MOX, with a reduction of over 95% with a lower credible interval above 90% on both studs. Mares on stud A had a FECR of 97.8% (CI: 93.3–99.9) for IVM and 98% (95.1–99.4) for MOX and stud B was 98.7% (94.4–100) for IVM. The strongyle ERP for the mares was between 7 and 10 weeks (Figs. 1B and 2B).

4. Discussion

The results of this study confirm the first case of ML resistance in cyathostomins infecting TB yearlings in the UK. The same population was also resistant to PYR and, given reports of widespread FBZ resistance in the UK it is likely that these cyathostomins had multidrug resistance to all anthelmintic classes licensed in horses. Resistance to MLs in cyathostomins has also recently been reported in the US following importation of horses from Ireland (Nielsen et al., 2020a), and in Australia (Abbas et al., 2021), suggesting that these three reports may not be isolated incidents. The intensive breeding practices on TBs studs and frequently applied anthelmintic treatments means that TBs could be described as sentinels of the wider equine population in alerting us to the risk posed by intensive treatment regimes. Thoroughbreds are very mobile hosts experiencing high levels of movement across the globe for races, sales and stallion visiting, bringing the opportunity for dissemination of resistant parasites.

Finding ML resistance in the UK is extremely concerning with regards to the future control of cyathostomins given that to the authors' knowledge, there are no plans for a novel equine anthelmintic to come to market. If we are no longer able to use MLs to effectively eliminate larval cyathostomins, the health, welfare and performance of horses may be comprised. We currently have very little evidence regarding the exact levels of infection which put a horse at risk of larval cyathostominosis; but if ML resistance spreads more widely we might expect there will be a concurrent risk of increased incidence of disease. In the absence of novel anthelmintics with alternative modes of action, we face the prospect controlling infection in cases of multi-drug resistance. It is encouraging that there are reports of combination de-worming being effective against cyathostomins with multiple anthelmintic resistance; e.g., a combination of MOX, oxibendazole, and PYR produced a 100% FECR in a cyathostomin population with both IVM and MOX resistance (Nielsen et al., 2020a), however oxibendazole and PYR were not individually tested in that study so it is possible that one or both of these drugs had high efficacy. Moreover, in populations where there is multiple anthelmintic resistance the first combination often yields an improved efficacy as susceptible worms are removed but subsequent treatments will likely have a lower efficacy (Scare et al., 2018). Combining anthelmintics has

Table 2
Faecal egg count reduction test results from 2021 to 2022.

Stud	Sample date	Group	Number sampled	Drug	% reduction 14 days post treatment	Pretreatment mean	1 week mean	2 week mean	3 week mean	4 week mean	5 week mean	6 week mean	7 week mean	9 week mean	10 week mean	ERP (weeks)
A	Mar-21	Y	11	IVM	40% (19.7–66.8)	767.5	*251	503	512	–	–	–	–	–	–	1
A	Apr-21	Y	9	IVM	36.4% (15.7–61.0)	503.3	*277.8	284.4	288.1	304.4	355	–	–	–	–	1
A	Jun-21	Y	11	MOX	72.6% (50.8–85.2)	324.5	*68	98.2	127.5	185.6	–	–	–	–	–	1
A	Aug-21	Y	10	PYR	80.8% (61.9–90.0)	829	–	*103.5	–	–	107.2	–	–	–	–	2
A	Jan-22	Y	55	IVM	76.3% (64.5–84.8)	917.3	–	*216.9	–	–	–	–	–	–	–	2
A	Apr-21	M	11	IVM	97.8% (93.3–99.9)	694.1	7	57.27	90	**25.4	–	**47.5	*116.4	–	–	7
A	July-21	M	10	MOX	98% (95.1–99.4)	484.5	7.5	16.5	27	–	–	42.2	–	–	*55	10
B	May-21	Y	14	MOX	99.9% (99.5–100)	282.9	–	0	–	3.93	–	*173.8	–	–	–	6
B	July-21	M	5	MOX	98.7% (94.4–100)	474	–	8	–	10	–	–	35	*59	–	9
C	Jun-21	Y	10	IVM	98% (95.4–99.2)	351	–	7	–	*111	–	–	–	–	–	4
C	Mar-22	Y	15	MOX	99.8% (98.4–100)	497.2	–	6.3	–	31.1	–	*103.3	–	–	–	6
D	Apr-22	Y	13	MOX	99.8% (98.4–100)	546.5	–	0.8	–	38.9	–	*266.2	–	–	–	6

(-No data point). * Is the timepoint in which the ERP is 10%. **Some individuals were missing. ***Percentage reduction 14 days post treatment (FECR) value gives the mean faecal egg count reduction and the 90% credible interval in parentheses. To determine the egg reappearance period (ERP), samples were collected every seven or 14 days until the mean FEC was at least 10% of the original value.

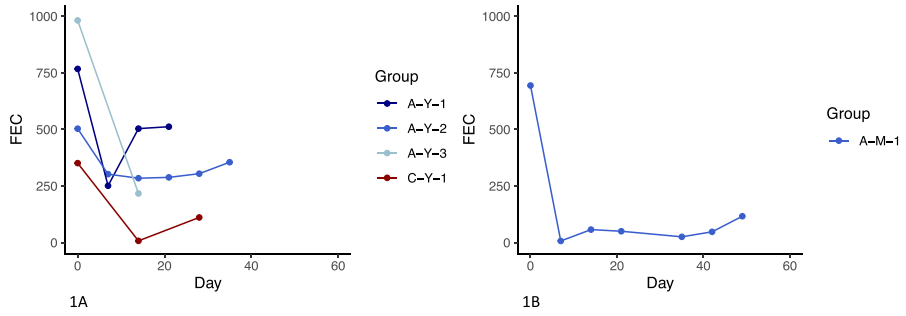

Fig. 1. Mean faecal egg count plotted for yearlings (A) and mares (B) after treatment with ivermectin for each timepoint sampled. A-Y-1 = stud A yearlings first sampling, A-Y-2 = stud A yearlings second sampling, A-Y-3 = stud A yearlings third sampling. C–Y-1 = stud C yearlings first sample. A-M-1 = stud A mares first sampling.

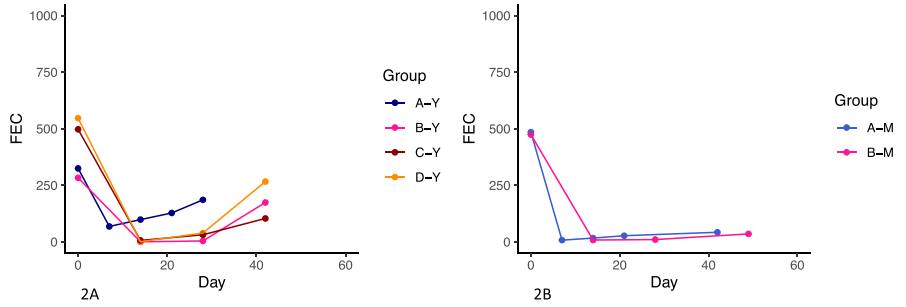

Fig. 2. Mean faecal egg count plotted for yearlings (A) and mares (B) after treatment with moxidectin for each timepoint sampled. Stud A-Y = stud A yearlings. Stud B–Y = stud B yearlings. Stud C–Y = stud C yearlings. Stud D-Y = stud D yearlings. A-M = stud A mares and B-M is stud B mares.

also been shown to be effective in sheep strongyles, attaining a higher efficacy than a single active alone (Learmount et al., 2012; Leathwick et al., 2012); however, in these studies at least one of the drugs in the combination had high efficacy, therefore further work is required to establish effectiveness of combining drugs when multi-drug resistance is present, such as we see here on stud A. Simulations have also shown that using combination treatments may be a useful tool to slow the development of anthelmintic resistance when it has not yet developed. For instance, by combining MOX with BZ to treat cyathostomin populations, models showed that, even with BZ efficacy as low as 50%, the development of resistance to MOX was slowed (Scare et al., 2020). It is interesting to note that in our study PYR had higher efficacy than MLs on stud A (the stud with ML resistance). Taken with the evidence outlined above, this highlights an important point that on yards where BZs and PYR are still somewhat efficacious they can potentially be utilised to increase longevity of the MLs.

In addition to smarter use of the available anthelmintics, there will inevitably be a need for increasing emphasis on environmental control, such as pasture management and parasite monitoring. Regular removal of faeces from the pasture has been shown to significantly reduce cyathostomin transmission by removing eggs from pasture before hatching (Proudman and Matthews, 2000; Corbett et al., 2014). Although this information is widely acknowledged, a study of 78 Irish Thoroughbred/sportshorse breeders found only 37.6% of the farms removed faeces from pasture (Elghryani et al., 2019). Interestingly, another study in which only 2% of the respondent's horses were kept on studs, 74.4% said they removed faeces from the pasture (Tzelos et al., 2017), suggesting that livery yards and private premises are practicing more responsible pasture management. This again highlights that stud farms are where uptake of best practice control methods needs to be focused, not least as they are responsible for large numbers of 'at risk' young animals kept at high stocking density.

As well as pasture control, targeted selective treatment (TST) has been advocated to slow the development of resistance by allowing for the development of refugia on pasture (Hodgkinson et al., 2019; Leathwick et al., 2019). Surprisingly, there are very little data proving that TST actually reduces development of anthelmintic resistance in horses; for example, there is only one epidemiological study demonstrating that TST regimes, combined with tighter biosecurity with new arrivals to a yard, resulted in a decreased risk of drug resistance (relative risk of 0.57, $p = 0.02$) (Sallé et al., 2017). Although we did not survey enough studs in this study to make a statistical comparison of parasite treatment protocols amongst studs, it is worth noting that Stud A was a large stud, with high stocking density and intensive interval drug treatment programs, whilst studs B, C and D were smaller scale studs using TST as part of their parasite management.

Yearlings on studs B, C and D did not appear to have resistance to the MLs, however, the yearlings on these studs all had shorter ERPs than the original published efficacies of nine-13 week for IVM (Boersema et al., 1996; Demeulenaere et al., 1997) and 16–22 weeks for MOX (Demeulenaere et al., 1997; Rolfe et al., 1998); with the observed ERPs of four weeks for IVM and six weeks for MOX being comparable to those reported in recent years (Lyons et al., 2010; Rossano et al., 2010; Tzelos et al., 2017; Molena et al., 2018). A reduced ERP has been suggested as an early indicator that resistance is developing, and there is some evidence that this is caused by L4 stages of cyathostomins being more resistant to treatment (Colglazier et al., 1977; Lyons and Tolliver, 2013), however this was refuted by two further studies (Lyons et al., 2010; Bellaw et al., 2018). Alternatively, shortened ERPs may be due to high mucosal burdens (particularly in young animals), which are not completely eliminated when treated (Bello and Laningham, 1994; Xiao et al., 1994; Bauer et al., 1998; Duncan et al., 1998). Overall, it is clear that further work is required to determine the causes of a shortened ERP (Nielsen et al., 2022). On stud A the efficacy of both IVM and MOX was

higher in parasites infecting older animals (i.e. mares) than it was against cyathostomin populations infecting yearlings. This has been observed previously when shorter ERPs were reported in youngstck compared to mares (Herd and Gabel, 1990; Relf et al., 2014). Younger animals are well documented as having a higher burden of cyathostomin infections and more reports of clinical syndromes than adult animals (Love et al., 1999; Chapman et al., 2003; von Samson-Himmelstjerna et al., 2009; Kornaś et al., 2010; Relf et al., 2013); but this does not necessarily mean that their cyathostomin populations should have different susceptibilities to anthelmintics. It is important to remember that the rate at which resistance develops is driven by the selection pressure exerted on the parasite population. There is greater selection for resistance in cyathostomin populations infecting yearlings as anthelmintics are used more frequently in youngstock in their first year of life. This, compounded by the fact that after weaning on stud A the yearlings were grazed separately from mares, and on the same permanent pasture over recurrent years, is one explanation for the difference between mares and youngstock. Yearling animals aquire their cyathostomin infection from their first grazing season and therefore are likely to be ingesting parasites from pastures contaminated with cyathostomin larvae exposed to high levels of anthelmintic in previous years' youngstock. This is supported by our observation that yearling cohorts in both 2021 and 2022 on stud A were both shown to harbour IVM resistant cyathostomin populations.

It is unlikely that our findings represent an isolated population of ML resistant cyathostomins in the UK, therefore, it is now imperative that measures are put in place to slow the spread of drug resistant cyathostomins, particulary in highly mobile TB populations. Ideally, this should include strict quarantine procedures including FECRTs for incoming horses on stud farms with combination worming treatments, as well as wide reaching surveillance and recording systems to determine the true extent of anthelmintic resistance on TB studs. Voluntary compliance for these approaches may result in a low uptake, hence these recommendations may require regulatory enforcement. This will be challenging to implement within the TB industry as it could ultimately interfere with the ability to sell animals with proven multi-drug resistant cyathostomin infections. Ultimately it is up to the racing industry to deal with the emerging problem of drug resistance parasites, but it is clear that further measures are essential to slow the development and spread of anthelmintic resistance in cyathostomins.

5. Conclusions

This study reports the first confirmed case of ML resistance in cyathostomin populations infecting UK TB yearlings. This finding is of great concern given the significant movement of TBs for both breeding and training, the intensity with which the MLs are used by TB studs and the lack of new anthelmintics coming to the market for equine use. Vigilant and regular monitoring using FECs and FECRTs to determine drug efficacies and taking active measures to prevent the dissemination of drug resistant parasites, for example by quarantining horses with pre- and post-treatment FECs, is required to determine the extent of resistance present in the UK and control the spread of resistant cyathostomins.

Declarations of competing interest

None

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Glossary

AR: Anthelmintic resistance
BZ: Benzimidazole
EPG: Eggs per gram
ERP: Egg reappearance period
FEC: Faecal egg count
FECR: Faecal egg count reduction
IVM: Ivermectin
ML-: Macrocyclic lactones
MOX: Moxidectin
PYR: Pyrantel
TB: Thoroughbred
WAAVP: World Association for the Advancement of Veterinary Parasitology