Anthelmintic Resistance in Equine Nematodes – A Review on the Current Situation, with Emphasis in Europe

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Abstract:
Since the introduction of the last equine broad-spectrum anthelmintic group in the 1980's, the investment in new drugs to control horse's parasites did not result in new advancements. These drugs allowed a very effective and extensive control of equine nematodes through successful interval dosing programs, firstly introduced in the 1960's. However, the widespread and indiscriminate use of anthelmintics in these intensive treatments have led to increasing resistance in the major equine nematodes. Reports of reduced effectiveness are virtually worldwide and repercussions in livestock production farms have already been seen.

Based on recent questionnaires about horse farm practices, preventive measures and international recommendations, it is clear that most of them are still not being widely implemented. It is also clear that these recommendations are outdated and new approaches must be considered to correctly tackle this rapidly evolving issue in horse management, as more accurate diagnostic methods are currently available, such as Mini-FLOTAC. This article intends to do a general review of the history and current situation of anthelmintic resistance in horses, with emphasis in Europe, as well as, how to diagnose and delay or even prevent its further development, mentioning new methods of diagnostic and directions in which to develop research.

Keywords: anthelmintic resistance, cyathostomins, Europe, horse, Mini-FLOTAC, nematodes.

1. Anthelmintic Resistance

The intestinal nematodes are genetically characterized by rapid rates of nucleotide sequence evolution because of their fast life cycles, which is exponential when considering their effective large population sizes, giving them a highly diverse genetic (Kaplan, 2004). Because of these features, it was only logical that strains of these parasites resistant to anthelmintic drugs would arise. These strains would be defined as populations where "the frequency of individuals able to tolerate doses of a compound is higher than in a normal population", with the capacity of transmitting this tolerance to newer generations, according to Prichard *et al.*. (1980). They also stated that this resistance could be directed to a particular drug compound with a similar mode of action (side-resistance) or other drugs of different anthelmintic groups (cross-resistance).

Nowadays, there are three broad-spectrum anthelmintic (AH) groups at the disposal of

Table 1 – Year of approval of broad-spectrum anthelmintic drugs in sheep and horses comparing to the first published report of its resistance. (Adapted from Kaplan, 2004).

Drug	Host	Year of initial drug approval[a]	First published report of resistance[b]
Benzimidazoles			
Thiabendazole	Sheep	1961	1964
	Horse	1962	1965
Pyrimidines			
Levamisole	Sheep	1970	1979
Pyrantel	Horse	1974	1996
Macrocyclic lactones			
Ivermectin	Sheep	1981	1988
	Horse	1983	2002
Moxidectin	Sheep	1991	1995
	Horse	1995	2003

[a]Approval in the United States of America
[b]The first published report did not normally coincide with the first clinical reports of inefficacy

veterinarians to treat grazing animals: the benzimidazoles (BZDs); imidazothiazoles (levamisole - LEV) and hydropyrimidines (pyrantel - PYR, morantel - MOR); and macrocyclic lactones (MLs) (ivermectin - IVM, moxidectin - MOX) (Kaplan, 2004; Coles *et al.*, 2006; Bowman, 2014). However, the appearance of anthelmintic resistance (AHR) was surprisingly fast when considering that the first reports go back to the late 1950's, with lack of effectiveness of phenotiazine against *Haemonchus contortus* in sheep (Prichard *et al.*, 1980; Kaplan, 2004). After the introduction of each new drug to the market, resistance has followed few years after (Table 1) and nowadays it is recognized as a major widespread problem in all livestock species (Prichard, 1994; Kaplan, 2004; Kaplan and Vidyashankar, 2012). A big part of the problem has been the lack of investment in new livestock AHs since the introduction of macrocyclic lactones in the 1980's, aside from monepantel use in sheep in few countries (Kaminsky *et al.*, 2008; Kaplan and Vidyashankar, 2012).

The intensive and regular deworming programs in place caused this alarming rate of AHR appearance in the great majority of farms, which impose a strong selection pressure for resistant strains of nematodes (Prichard *et al.*, 1980). Related to this, another important factor is the deworming of animals not heavily infected, reducing the population of parasites that are not exposed to AHs. This population is called refugia and includes not only parasites in non-dewormed hosts, but also free-living stages of parasites (like the ones on the pasture) and parasitic stages in the host that are not affected by the used AHs (like encysted parasites in the large intestine wall) (Wyn, 2001; Kaplan, 2004; Kaplan and Nielsen, 2010). Currently, refugia is considered as important to tackle the advancements in the AHR problem, as spared and alternate use of the drugs themselves (Wyn, 2001; Besier, 2012). Furthermore, other factors influence the appearance and advancement of resistance like fecundity of female worms, lifespan of mature worms, survival of free-living stages in the environment and manner of inheritance of resistance traits, concerning the parasite's biology; and levels of innate and acquired immunity and behavioral differences affecting exposure rates of the hosts (Churcher *et al.*, 2010).

1.1. Diagnosing resistance

The impact of AHR is rapidly becoming visible as more farms shut down their production due to the presence of multiple drug resistance nematodes (Sarginson *et al.*, 2005). The diagnosis of AHR has been thoroughly reviewed over the years in order to find suitable ways to detect it in time to prevent these situations. Today, these diagnostic methods are divided in molecular techniques and more evidence-approached techniques, extensively reviewed by Coles *et al.* (2006). This separation is extremely important as the former detect the presence of genetic resistance (alleles) in the population, which evolves slowly over time, whereas the latter detect the phenotypic manifestation of resistance in a host population that can appear suddenly (Kaplan and Vidyashankar, 2012). Despite molecular techniques like PCR being useful as sentinels for

Table 2. Summary of reported resistance worldwide of the main equine nematodes to broad-spectrum anthelmintics. (Adapted from Nielsen et al., 2019).

Drug Class	Cyathostomins	Large strongyles	*Parascaris* spp.	*Oxyuris equi*
Benzimidazoles	Widespread	None	Early indications	None
Pyrimidines	Common	None	Early indications	None
Macrocyclic lactones	Early indications	None	Widespread	Early indications

the detection of rising resistance, they still have limitations as they are allele-specific and drug-specific, with only benzimidazoles specific alleles established (Taylor, Hunt and Goodyear, 2002) and don't quantify yet the influence of multiple alleles to the clinical manifestation of resistance (Kaplan and Vidyashankar, 2012).

Other than molecular techniques, multiple approaches have been developed, either *in vitro* or *in vivo*. The first group includes methods like egg hatch assay for BZDs; larval paralysis and motility test for BZDs, PYR, LEV and MOR; larval development test for BZDs, LEV and IVM; among other less common techniques. The *in vivo* techniques are mostly limited to the Faecal Egg Count Reduction Test (FECRT), with comparison of Eggs per Gram (EPG) in Faecal Egg Counts (FEC) prior and 14 days as an average after treatment with AHs (Coles *et al.*, 1992; Taylor, Hunt and Goodyear, 2002; Coles *et al.*, 2006). Nonetheless, because all *in vitro* techniques imply a laboratorial assessment of AHR, FECRT has been assumed as the practical gold standard to define resistance at the farm level in all livestock species and it can only be interpreted for the population and not individuals (Kaplan and Vidyashankar, 2012).

2. Resistance in equine nematodes

In the 1960's, the equine health management and welfare changed forever with the introduction of BZDs as an AH for horses, generating a new epidemiological approach to parasite control in this domestic species. This new system was designed to control the infections by *Strongylus* spp., especially *Strongylus vulgaris*, based on an interval dose system of 6-8 weeks (Kaplan, 2002), which prevented the maturation of any intra-luminal larvae development. The success of this deworming program was recognized worldwide in the equine community (Lyons, Tolliver and Drudge, 1999) and by 1983 with the introduction of ivermectin as a larvicidal drug of *Strongylus* spp. migrating larvae, these parasites were already considered uncommon and dissociated from equine colic development as a cause, maintaining this status until today (Kaplan, 2002).

Therefore, the main parasites of managed horses have changed, with cyathostomins turning the most important pathogenic parasites nowadays (Love and Duncan, 1991; Lyons, Tolliver and Drudge, 1999). However, the widespread interval dose programs directed for *Strongylus* spp. continued, even with their stated decreasing prevalence. These dewormings used rotation of the equine AHs, which currently are the same three classes described before (Gokbulut and McKellar, 2018). As a result, as seen in Table 2, for the past decades, there have been continuous reports of growing resistance to all classes of AHs worldwide in all major equine nematode groups, particularly cyathostomins, *Parascaris* spp. and *Oxyuris equi* (Kaplan, 2004; Kaplan and Nielsen, 2010; Cernea *et al.*, 2015; Nielsen *et al.*, 2019).

2.1. Diagnosing resistance in horses

In horse management, the practical gold standard at field level to analyse these resistances is FECRT, assessing the AH effectiveness in reducing the FECs , as previously described (ESCCAP, 2019; Nielsen *et al.*, 2019). AAEP guidelines by Nielsen *et al.* (2012a) suggest the inclusion of at least 6 horses in a FECRT, preferably the ones with the highest FEC, and that these horses have not been previously dewormed for at least 8 weeks. With these requirements fulfilled, it is possible to evaluate the efficacy of the AH drugs 14 days after they are used and FEC reduction thresholds have been established (Table 3). Below these cut-off values, resistance of the parasite population in question can be inferred.

Even though FEC of mature horses are normally consistent overtime (Nielsen, Haaning and Olsen, 2006; Carsten, Larsen, Ritz and Nielsen, 2013), many factors influence FEC and therefore FECRT. Among them, non-uniform distribution of the eggs in the faeces, storage of the faecal samples,

Table 3. Thresholds of FECRT results to determine the presence of anthelmintic resistance to equine broad-spectrum anthelmintics. (Adapted from Nielsen et al., 2019).

Anthelmintic	Expected efficacy if no resistance	Susceptible (no evidence of resistance)	Suspected resistance	Resistant
Fenbendazole/Oxibendazole	99%	>95%	90-95%	<90%
Pyrantel	94-99%	>90%	85-90%	<85%
Ivermectin/Moxidectin	99.9%	>98%	95-98%	<95%

Table 4. ERP of equine broad-spectrum anthelmintics when the drug is fully effective on cyathostomins. (Adapted from Nielsen et al., 2019).

Anthelmintic	Usual ERP when drug is effective	ERP when drug was first introduced	ERPs on farms with emerging resistance
Febendazole/Oxibendazole	4-5 weeks	6 weeks	-*
Pyrantel	4-5 weeks	5-6 weeks	-*
Ivermectin	6-8 weeks	9-13 weeks	3-5 weeks
Moxidectin	10-12 weeks	16-22 weeks	4-6 weeks

*Resistance so commonly reported that ERPs have not been measured

egg loss during technical processing (»30% in the modified-Wisconsin method, for example), the coprological method used and its sensitivity and statistical processing of the data, are some of the most important factors (Vidyashankar, Hanlon and Kaplan, 2012; Ballweber, Beugnet, Marchiondo and Payne, 2014).

The FECRT can be easily employed for the assessment of efficacy of all drug classes against nematodes, e.g. strongyles and *Parascaris* spp., being less meaningful for *O. equi* (ESCCAP, 2019). The method used to perform FECRT is extremely important in detecting AHR, as a more sensitive technique will provide more accurate FEC and consequently its reduction percentage. The most recent coprological method presented to this purpose was the Mini-FLOTAC (Cringoli *et al.*, 2013). It is a flotation technique that uses its own measuring container, sampling kit and filter built in (Fill-FLOTAC) with its unique reading disk (Mini-FLOTAC). These characteristics make this method usable virtually anywhere, without the need of expensive laboratory equipment, apart from a microscope. There are several protocols described already, available online from the Parasitology Department of the University of Naples, for humans and different animal species, proving its accuracy and precision (Went, Scare, Steuer and Nielsen, 2018). The current recommended technique to detect AHR is the McMaster method (Duncan *et al.*, 2002; Coles *et al.*, 2006).

However, more and more studies are determining the Mini-FLOTAC as the most sensitive, accurate and usable in the daily practice of horse farms (Britt *et al.*, 2017; Dias de Castro *et al.*, 2017; Noel, Scare, Bellaw and Nielsen, 2017; Scare *et al.*, 2017; Paras, George, Vidyashankar and Kaplan, 2018), possibly making it a better option to perform FECRT. If a continuous monitoring of FECs is maintained, another parameter that can indicate the rising of AHR in a population is the Egg Reappearance Period (ERP), the time it takes for FECs to reassume significant values of egg shedding after deworming (Nielsen *et al.*, 2019), which was already established for cyathostomins (Table 4). A shorter ERP than the recorded one is suggestive of increasing AHR.

2.2. Anthelmintic resistance in equine nematodes

In Europe and in the United States of America (USA), AHR of *Parascaris* spp. to macrocyclic lactones has been recently documented (Schougaard and Nielsen, 2007) and extensively reviewed (Reinemeyer, 2009), pointing out the current effectiveness of only benzimidazoles against these parasites. Something to consider when analysing the growing resistance of *Parascaris* spp., is that it is a dose-limiting parasite (DLP) for most equine AHs, which means that, in order to kill it, a higher dosage must be used [36]. However, because most equine AHs

Table 5. Classification of horses according to their faecal egg count and their respective proportion in the population. (Adapted from Nielsen et al., 2019).

Egg count level		Adult population[a]
Low shedders:	0-200 EPG	50-75%
Medium shedders:	200-500 EPG	5-15%
High shedders:	>500 EPG	10-30%

[a]These values are only estimates and the actual percentage of horses in each category will vary among farms depending on a multitude of factors.

today are considered broad-spectrum, using the recommended dosage will leave room for DLP's to grow resistant.

For cyathostomins, however, the problem is much bigger, as these parasites have been documented to be resistant to all classes of broad-spectrum drugs, except for macrocyclic lactones (Kaplan, 2004; Matthews, 2008; Corning, 2009; Bellaw *et al.*, 2018). Their resistance against benzimidazoles has been constantly recorded and reviewed (Matthews, 2008; Corning, 2009) and Bellaw *et al.* (2018) recently evidenced that these drugs can no longer be considered as effective against these parasites due to the widespread of resistant strains. Another important studied AH was PYR, which effectiveness was also evidenced to be decreasing (Kaplan, 2004).

Nonetheless, PYR resistance is mainly recorded in the USA and Canada, the only two countries where a daily oral dosage of PYR in the diet is considered a normal approach to horse management. Some suggestions have aroused that this might be the reason behind that increasing resistance and that this practice should be discontinued (Kaplan and Nielsen, 2010). Finally, the only still fully effective AH in horses against cyathostomins are macrocyclic lactones. However, even signs of rising resistance to this class have been pointed out, with commonly found shorter ERPs, from 8 weeks to 4 weeks (Lyons, Tolliver and Collins, 2009; Bellaw *et al.*, 2018; Molena *et al.*, 2018).

3. Delaying and preventing resistance

As seen, the extensive use of intensive chemical deworming techniques has been very short-sighted (Kaplan, 2004) and the approach to equine nematodes' control has to be reviewed and it needs to be integrated with non-chemical methods. Such methods can include selective treatments, correct management of the pasture and its hygiene, biological control with nematophagous fungi and quarantine of new animals.

3.1. Selective anthelmintic treatment programs

The use of anthelmintics in equine management should not be abolished, but reduced and conscious, instead. With the confirmation of AHR around the world, a new approach to AHs emerged based on two principles: a) the egg shedding of mature horses is consistent (Nielsen, Haaning and Olsen, 2006; Scheuerle *et al.*, 2016); b) 80% of the eggs shed and contaminating the pasture are resulting from just 20% of the population (Kaplan and Nielsen, 2010), or even a lower percentage of hosts (Lester *et al.*, 2013; Relf *et al.*, 2014). These two statements are the justification for the new Selective Anthelmintic Treatment (SAT) or Targeted Selective Treatment (TST) programs and intend to use refugia as the buffer for the onset of AHR (Kaplan, 2004; Kaplan and Nielsen, 2010; Besier, 2012; Pfister and van Doorn, 2018). Once in place, SAT or TST programs attempt to: 1) understand the epidemiology of the present nematodes; 2) determine which drugs are effective in the farm; 3) use the right AH for the correct parasite developmental stage at the appropriate time of the year; 4) determine which horses require less or more frequent treatment; and 5) evaluate the overall success of parasite control (Kaplan and Nielsen, 2010).

According to the European Scientific Counsel Companion Animal Parasites (ESCCAP) the SAT/TST approach should only be recommended for adult horses and exclusively designed for the control of small strongyles (ESCCAP, 2019). In this type of deworming programs, not all adult horses in the farm should be dewormed for 12 weeks and after that, faecal samples of every horse should be analysed to obtain its FEC. Then, according to a certain cut-off value, only the horses exceeding it (medium and high shedders) should be treated

Table 6. Adulticidal and larvicidal action of broad-spectrum anthelmintics in main equine nematodes. (Adapted from Decision Tree Horse, http://www.parasietenwijzer.nl/eng/ horse/GB_DesicionTreeHorse.html, accessed in June 2019).

	Benzimidazoles	Pirymidines	Macrocyclic lactones
Ascarids	Adult and larval stages and worm eggs	Adult stages	Adult and larval stages
Cyathostomins	All stages and worm eggs**	Adult stages (efficacy often less than 90%)	Adult and immature stages * (not L3 or encapsulated larvae)
Strongylins	All stages and worm eggs**	Adult stages (efficacy often less than 90% and particularly *S. edentatus* not very sensitive)	Adult and all larval stages (except for *S. equinus* only adult stages)

*MOX is partially effective against encapsulated larvae and has a residual effect for two weeks; ** To obtain a high efficacy against larval stages (in the mucosa or migrating) it is often recommended to treat with fenbendazole for 5 consecutive days.

with AH and the others (low shedders) should be left untreated to act as refugia for the population. This cut-off value as long been discussed in the equine community with some statements being made that it should be 200 EPG, as shown in Table 5 (Kaplan and Nielsen, 2010; Pfister and van Doorn, 2018; Rendle *et al.*, 2019). However, a study reports that a range of up to 500 EPG should be used, as it holds a better relation between FEC and worm burden (Nielsen *et al.*, 2010).

This range should also be considered according to the risk of infection in the farm in question (Table 7), with farms with lower risk being able to tolerate horses with 500 EPG as threshold (Rendle *et al.*, 2019). Faecal egg counts should be performed in moderate climates during grazing season, from March to September (until October/November in Mediterranean countries), preferably each 8-12 weeks (Rendle *et al.*, 2019) and they should be performed in triplicates every time, as it reduces the variability inherent to this method (Nielsen, Haaning and Olsen, 2006; Vidyashankar, Hanlon and Kaplan, 2012).

In the first year of the implementation of SAT/TST programs, the parasite monitoring may seem expensive and labouring but, since adult horses maintain their egg shedding consistent, keeping up monitoring and control of high shedders is much easier afterwards(Pfister and van Doorn, 2018). The medium and high shedders should be dewormed with an effective AH and FECRT is recommended to be performed, at least annually, after the ERP considered for the drug used in order to evaluate the appearance of AHR (Churcher *et al.*, 2010; Rendle *et al.*, 2016; Nielsen *et al.*, 2019). Taking into consideration the current status of AHR and the effectiveness of different AHs (Table 6) (Faculty of Veterinary Medicine of the University of Utrecht, 2019), the most recommended drugs to use in these high shedders are IVM and PYR (Rendle, 2019). Furthermore, rotation of AHs should not be encouraged as it has been proven not to delay AHR and there are few effective drugs to rotate (Kaplan and Nielsen, 2010; Leathwick, 2013; Shalaby, 2013; Pfister and van Doorn, 2018). In order to prevent the further development of AHR in cyathostomins, treatments are most effective during winter (Sauermann, Nielsen, Luo and Kaplan, 2019).

SAT/TST programs promote a logical, spared and justified application of AHs and provide a greater amount of refugia in the parasite population (Nielsen, 2012; Pfister van Doorn, 2018). Consequentely, treating only high shedders, the contamination of the pasture will be significantly lower, as they are the ones who contribute the most for it and the surviving parasites with resistant genes will be diluted because of the greater refugia size (Besier, 2012).

It is important to state, however, that the true aim of SAT/TST programs is to delay or even prevent the development of AHR and that clinical parasitic disease may still occur from pasture infection, namely by infection with *Strongylus* spp., particularly *Strongylus vulgaris*, which will increase the risk of horse colic. Thus, the integrated use of AHs and non-chemical control methods must be put in place after a good balance of the advantages and disadvantages of SAT/TST for each horse, farm and region, according to local and regional knowledge of horse parasite epidemiology, namely if no *S. vulgaris* L3 larval

Table 7. Assessment risk of infection from pasture considering various factors and practices in horse stud farms. (Adapted from Rendle et al., 2019).

Low risk	Moderate Risk	High risk
Repeated negative FEC	Low/moderate FEC	High FEC
5–15 years old	>15 years old	<5 years old
Faecal collection > twice per week	Sporadic faecal collection	No faecal collection
Good pasture management	Moderate pasture management	Poor pasture management
Stable population	Occasional movement of the animals	Transient population
Low stocking density	Medium stocking density	High stocking density
No young stock		Grazing with young stock
Effective quarantine		No quarantine
No history of parasitic disease		History of parasitic disease
No history of colic		History of colic
		AHR identified on property by ECRT

stages are found in monitoring faecal cultures (Besier, 2012; ESCCAP, 2019).

3.2. Pasture management

Pasture hygiene is very effective in preventing re-infection and it is of great use when considering a selective grazing species like the horse. The removal of faeces twice weekly was shown to be a valuable practice in reducing significantly the contamination of the pasture and even more effective than treatment with AH (Herd, 1990).

Furthermore, while grazing, horses determine defecation areas (roughs) where grazing is avoided and herbage is normally dense and feeding areas (lawns) (Reinemeyer and Nielsen, 2018), making it easier to select areas for those cleanings. Because of this, horses are a grazing species that can greatly benefit from faecal removal as a non-chemical worm control measure, but also as a way of increasing grazing areas in paddocks (Herd, 1990).

Other pasture management methods to interrupt parasite transmission and reduce the need of using AHs, include harrowing, rotation or mixed grazing. Harrowing pastures to break up faecal pellets and expose free-living parasitic stages can also be of valuable use to control parasite transmission in sub-tropical climates, such as in southern Europe, due to the higher temperatures registered in the summer (Reinemeyer and Nielsen, 2018).

Pasture rotation can also be applicable to decrease the contamination of grazing areas in temperate climates and a 6-week grazing period per pasture with 18 weeks of rest was shown to be effective (Hernández *et al.*, 2018).

Mixed or alternate grazing of pastures with ruminants has also been described to reduce strongyle infection in horses, but care must be taken as some gastrointestinal nematodes are sharer by both hosts (Reinemeyer and Nielsen, 2018).

3.3. Nematophagous fungi

A new promising biological control of worms has been extensively studied recently consisting of feeding spores of nematophagous fungi to horses, with the purpose of controlling the free-living parasitic stages. The fungal chlamydospores are able to survive the intestinal tract of the horse and develop in the faecal environment, developing hyphae that trap and, consequently, kill larvae (Larsen, Nansen and Hendriksen, 1995). *Duddingtonia flagrans* and *Monacrosporium thaumasium* or *Mucor circinelloides* are some fungi species that have demonstrated effectiveness in reducing equine infective larvae on pasture, with their larvicidal and ovicidal effect (Tavela *et al.*, 2011; Buzatti *et al.*, 2015). Reduction rates of 90% and higher have been reported (Fernandez and Larsen, 1997; Buzatti *et al.*, 2012) and longer periods of unneeded treatment compared to the use of AHs have been documented (Hernández *et al.*, 2016), making this one of the most promising measures in preventing further development of AHR, namely during quarantine, in the stable or on the pasture.

3.4. Quarantine

Basic and careful hygiene and quarantine measures, both for horses in stables and on pasture are important to reduce the infection risk and consequently the need for treatment. According to ESCCAP, to prevent introduction of new parasite species and/or resistant parasite populations, each horse recently introduced to a farm should be quarantined and treated after arrival. Therefore, the animal should only be moved to pasture after a FEC performed five days post treatment has confirmed that the horse is negative concerning worm eggs and that deworming was successful (ESCCAP, 2019).

4. Current situation – farm practices and legislation

Even with all this information available about AHR, the integrated approaches to deworming horses have slightly changed. In several recent questionnaires directed to horse farms, recommendations to prevent it are poorly implemented. The chemical approach to deworming horses appears to have changed, with farms decreasing AH administrations from as high as 6 per year (Lloyd *et al.*, 2000) to 2-3 doses a year (Hinney *et al.*, 2011; Nielsen *et al.*, 2018), as a growing percentage of them adheres to SAT/TST programs (Stratford *et al.*, 2014). In some regions, however, 4-5 doses of AHs are still being given (Elghryani, Duggan, Relf and de Waal, 2019). The rotation of these drugs seems to be also disappearing, with more horse farms stopping the simultaneous use of three drug classes per year (Lloyd *et al.*, 2000) and relying only in macrocyclic lactones to deworm their animals (Hinney *et al.*, 2011; Stratford *et al.*, 2014; Nielsen *et al.*, 2018). However, there are still some farms using benzimidazoles, despite all the recommendations to avoid them (Elghryani *et al.*, 2019; Fritzen, Rohn, Schnieder and von Samson-Himmelstjerna, 2010). Another concerning fact is that FECs are still not widely implemented as part of the parasite control programs as expected (Relf, Morgan, Hodgkinson and Matthews, 2012; Nielsen *et al.*, 2018; Scare *et al.*, 2018; Elghryani *et al.*, 2019), with some farms having never performed a FECRT (Fritzen *et al.*, 2010).

Pasture management seems to be increasing in horse farms (Nielsen *et al.*, 2018), with about 40% of them removing faeces twice weekly (Hinney *et al.*, 2011; Stratford *et al.*, 2014; Elghryani *et al.*, 2019), but some still don't apply this practice (Fritzen *et al.*, 2011). Sub-dosing of AH drugs is also a point of interest, since some horse farms still use imprecise methods of weighting animals (Hinney *et al.*, 2011; Fritzen *et al.*, 2011; Relf *et al.*, 2012; Elghryani *et al.*, 2019), while other are more prone to determine more precise weights before deworming (Stratford *et al.*, 2014).

Perhaps the most concerning subject is that the great majority of horse owners are unaware of AHR in their farms and they are not very concerned about it (Stratford *et al.*, 2014). This may indicate a distancing between horse owners and veterinarians, since AHs are so easily bought and administered to horses nowadays, in contrast to the past (Kaplan and Nielsen, 2010). Nonetheless, legislation has already been introduced in Europe to turn equine anthelmintic administration prescription-only, to prevent the further development of AHR. Denmark was the first country to adopt this strategy in 1999 (Nielsen, Monrad and Olsen, 2006), with significantly good results, as the use of AHs as gotten lower along with EPG, increasing strongyles and AHR surveillance (Nielsen *et al.*, 2014; Becher *et al.*, 2018). After this change, the European Union followed with directives to apply restrictions on anthelmintic administration in livestock (EU, E.P.a.o.t.C, 2001; EU, E.P.a.o.t.C, 2006), and currently the Netherlands, Finland, Sweden, Austria and Germany, all have this approach to equine AHs (Becher *et al.*, 2018). As a consequence of these changes, *Strongylus* spp. appears to be reemerging as interval dosing treatments are discontinued (Nielsen, 2012; Tydén *et al.*, 2019), evidencing the importance of non-chemical approaches to parasitic control and the regular parasitological monitoring of horse farms.

According to ESCCAP, as a final remark towards a good level of parasite control, together with prophylaxis and management of AHR, the preventive measures, routine monitoring and regular deworming practices should be clearly explained to the horse owners by veterinarians, veterinary nurses and other animal health professionals. Namely, parasite control programs need to be fitted to each individual horse farm or facility and should be discussed and developed under veterinary supervision (ESCCAP, 2019).

Conclusion

As seen here, anthelmintic resistance is perhaps the main problem in horse management nowadays and it needs to be responsibly approached. The prescription of an anthelmintic treatment has to be carefully considered in horse farms and needs to discriminate the horses that truly would benefit from it. Resistance should be addressed by each equine veterinarian in their routine practice with the endorsement of SAT/TST programs in adult horses, thoroughly explained to horse owners to communicate the real dimension of the problem. With this, more measures that are non-chemical can be actively taken to control worms in farms, as the ones presented here, and delay the development of anthelmintic resistance. Investment in new methods to diagnose rising resistance in horses should be encouraged and ways to put them economically at the reach of farms should be further studied. In addition, a different approach to detect anthelmintic resistance at the farm level must be used, as new and more sensitive methods to perform FECRT are currently available, such as the presented Mini-FLOTAC. The repeatability and reliability of these new techniques are constantly being addressed in recent years, but further studies on their performances are needed.

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