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Review Article

Practical aspects of equine parasite control: A review based upon a workshop discussion consensus

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Summary

Development of resistance of several important equine parasites to most of the available anthelmintic drug classes has led to a reconsideration of parasite control strategies in many equine establishments. Routine prophylactic treatments based on simple calendar-based schemes are no longer reliable and veterinary equine clinicians are increasingly seeking advice and guidance on more sustainable approaches to equine parasite control. Most techniques for the detection of equine helminth parasites are based on faecal analysis and very few tests have been developed as diagnostic tests for resistance. Recently, some molecular and *in vitro* based diagnostic assays have been developed and have shown promise, but none of these are currently available for veterinary practice. Presently, the only reliable method for the detection of anthelmintic resistance is a simple faecal egg count reduction test, and clinicians are urged to perform such tests on a regular basis. The key to managing anthelmintic resistance is maintaining parasite refugia and this concept is discussed in relation to treatment strategies, drug rotations and pasture management. It is concluded that treatment strategies need to change and more reliance should now be placed on surveillance of parasite burdens and regular drug efficacy tests are also recommended to ensure continuing drug efficacy. The present review is based upon discussions held at an equine parasite workshop arranged by the French Equine Veterinary Association (Association Vétérinaire Equine Française, AVEF) in Reims, France, in October 2008.

Introduction

Equine parasite control remains a complex and constant challenge for both owners and their veterinary advisers. Horses are infected by a wide range of helminth species and differences in life cycles,

epidemiology, pathogenicity and drug susceptibility makes it increasingly challenging to design effective and sustainable parasite control programmes. Decades of regular and intensive anthelmintic treatments have led to increasing levels of drug resistance to most of the currently available equine anthelmintics (Kaplan 2002; Kaplan *et al.* 2004). In the case of equine nematode parasites, resistance has been documented to the 3 major drug classes marketed for horses and it is not known if or when any new drug classes with different modes of action will be introduced for use in the horse.

Given this situation there is a general consensus among veterinary parasitologists that more sustainable approaches to parasite control need to be developed and refined, and that these would involve increased levels of surveillance of both parasite infections and drug efficacy. Scientists involved with research and education in the field of equine parasitology constantly encounter questions from practitioners and horse owners relating to parasite control. Simple recommendations are often expected, but the provision of such recommendations has become increasingly complex and difficult, and there may even be some differences of opinion between the scientists themselves.

At the 2008 meeting of French equine veterinary practitioners (Association Vétérinaire Equine Française, AVEF, 2008), the organisers arranged a one-day workshop focusing on equine parasite control. French equine practitioners were invited to submit questions within the field of equine parasitology and a panel of specialists from a number of different European countries were invited to discuss and answer these questions. This created a unique and rare opportunity for the participants to discuss relevant practical issues relating to parasite control and to try to reach some level of consensus. A representative selection of the questions submitted for this workshop and the answers provided by the expert panel is provided in Table 1.

This review highlights the main conclusions from the workshop and provides a literature background in support of these

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TABLE 1: Selected questions submitted by French veterinary practitioners and answers provided by the panel members at the Equine Parasitology Workshop in Reims, France 2008

Question	Answer
Which parasites can be detected with a plain egg count analysis?	Strongyle eggs (species differentiation requires larval culture) <i>Parascaris equorum</i> <i>Strongyloides westeri</i> <i>Eimeria leuckarti</i> <i>Oxyuris equi</i> (occasional finding) <i>Anoplocephala perfoliata</i> (occasional finding)
Which species of parasites can be detected with serological tests?	<i>Anoplocephala perfoliata</i> (serum antibody ELISA) <i>Fasciola hepatica</i> (equine test under validation)
Have any specific haematological and biochemical parameters been associated with gastrointestinal parasitosis?	Low plasma protein and albumin/globulin fractions below 0.7 have been associated with larval cyathostomiasis. Eosinophil counts are poorly understood and are generally inconsistent with parasite infections. No haematological or biochemical finding is pathognomonic for parasite infection
Does anthelmintic drug class rotation help in preventing resistance?	There is no current evidence supporting this. On the contrary, drug rotation does not appear to affect development of drug resistance
Which pasture management techniques can reduce pasture contamination?	Pasture hygiene, when performed at least weekly or biweekly Mixed or alternate grazing with ruminants Mid-summer movement Pasture rotation (in warmer climates)
How long should a horse be stabled for after deworming, to prevent contamination of a 'clean' pasture?	There is no scientific reason to do this after strongyle treatment. However, in the case of <i>P. equorum</i> treatment, stabling for a few days could be considered to lower contamination of the environment
Are there any scientifically proven methods of obtaining an accurate weight measurement in the absence of scales, irrespective of the horse's size?	Weight-band measurements have been evaluated in several studies. In most horse breeds these have proven reliable, although smaller breeds can be more difficult to measure. It is often accepted to dose according to the estimated bodyweight plus 15%
Does the formulation of the active ingredient of an anthelmintic have any impact on its efficacy?	Yes, drugs formulated for other host species and other route of entries should not be used in horses. The formulation affects the pharmacokinetics of the drug and its metabolites and dosage cannot be extrapolated from other host species
Is fenbendazole (10 mg/kg bwt daily for 5 days) effective for the treatment of horses known to host benzimidazole-resistant cyathostomins?	Although there may be a contemporary effect, this treatment regimen will eventually prove ineffective as well
What are the current recommendations regarding preoperative deworming and is there any scientific evidence of a beneficial effect on the risk of post operative complications?	There have been documented increased risks of colic and diarrhoea after treating heavy parasite burdens. Thus, in case of elective surgery, anthelmintic treatments should be done well ahead of the procedure (i.e. 1–2 weeks). In case of acute surgery, treatments should wait until the horse is stabilised and has regained normal gastrointestinal function and motility

conclusions. The paper is structured as follows: 1) a review on currently available diagnostic methods in equine parasitology, in each case indicating their perceived strengths and limitations; 2) a brief summary of potential future diagnostic tests; 3) a discussion of anthelmintic resistance (AR), including the current situation, diagnostic methods for the detection of resistance and recommended management options; and 4) a description of various practical management procedures that can be employed in the control of parasites in the horse.

The major significant parasites that can infect horses are listed in Table 2. Textbooks in veterinary parasitology may be consulted for more detailed information.

Diagnostic methods

Faecal egg counts

Faecal egg counts can be measured by numerous modifications of the well-known McMaster (Gordon and Whitlock 1939), Stoll (Stoll 1923) and Wisconsin (Cox and Todd 1962) techniques. All of these are flotation methods where samples of faeces are suspended in a fluid with a specific gravity that allows eggs to float to the surface, where they can be detected microscopically. Recently published methods, such as the FECPAK (Presland *et al.* 2005) and FLOTAC (Rinaldi *et al.* 2007) are based on the McMaster technique.

Many different flotation media have been used and the choice varies between laboratories, but typically they are based on solutions of zinc or magnesium sulphate, sodium chloride and/or

sugars such as sucrose. Some methods use passive flotation (Gordon and Whitlock 1939; Presland *et al.* 2005), while others use flotation obtained by centrifugation (Cox and Todd 1962; Roepstorff and Nansen 1998; Rinaldi *et al.* 2007). There are numerous modifications of basic egg counting techniques and every laboratory tends to have its own preferred method. Although this

TABLE 2: Parasites infecting horses under European conditions and their most common clinical manifestations

Parasite	Clinical relevance
Cyathostomins	Ubiquitous parasite group Larval cyathostomiasis, diarrhoea, weight loss
<i>Parascaris equorum</i>	Very common in foals and yearlings Weightloss, diarrhoea, small intestinal impaction/colic and/or perforation
<i>Strongylus vulgaris</i>	Now very rare in well-managed equine establishments. Thromboembolic colic
<i>Anoplocephala perfoliata</i>	Very common in grazing horses. Occasional ileal impaction, spasmodic colic
<i>Dictyocaulus arnfieldi</i>	Rare, most often associated with horses grazing with donkeys Variable clinical signs
<i>Oxyuris equi</i>	Common Perianal irritation, pruritus
<i>Gasterophilus</i> spp.	Very common No documented clinical manifestations due to larval bots in the stomach
<i>Strongyloides westeri</i>	Rare. Has been associated with diarrhoea in very young foals
<i>Habronema</i> and <i>Draschia</i> spp.	Rare. Mild gastritis, summer sores
<i>Fasciola hepatica</i>	Rare. Clinical implications unknown

can be confusing, in reality they are only modifications of the same principle and the most important aspect is the individual detection limit, some techniques being more suitable for detecting low egg counts than others. It is also recommended that laboratories routinely participate in procedures to validate their techniques. A simple approach would be annual ring tests, where faecal samples from a number of horses are analysed by a number of different laboratories. At least one of these should have a reference status, such as a university or a national diagnostic laboratory.

Distinct egg morphologies allow diagnosis of some important equine helminth infections. These include *Parascaris equorum* and *Strongyloides westeri*. Occasionally, eggs of the equine pinworm, *Oxyuris equi*, may be present but, as a rule, these are found around the anus and are not shed in the faeces. Exceptionally, eggs of *Fasciola hepatica* or *Dicrocoelium lanceolatum* may be identified. Eggs of anoplocephalid tapeworms are easily distinguishable when found on faecal examination, but specific modifications of egg count techniques are required specifically to detect tapeworm eggs. Proudman and Edwards (1992) validated a tapeworm-specific egg counting technique, which is based on a Stoll-like method utilising centrifugation-flotation to achieve an overall diagnostic sensitivity of 0.61.

Oocysts of the protozoan parasite *Eimeria leuckarti* may also be identified on faecal examination, especially in foals. The significance of this parasite is not known.

Strongyle faecal egg counts (FECs) are subject to a high degree of variation due to an uneven distribution in the faeces and fluctuations of up to 50% between repeated counts have been reported (Uhlinger 1993). In addition, studies in cattle (Michel 1968, 1969; Brunson 1971) and sheep (McKenna 1981; Reinecke and Groeneveld 1991; Stear *et al.* 1995) suggest that there is a poor correlation between worm numbers and egg counts; although there have been no quantitative studies in horses, a similar relationship is suspected to exist (Duncan 1974). Despite these apparent drawbacks, there appears to be a strong level of consistency in levels of egg shedding in individual horses over time (Gomez and Georgi 1991; Döpfer *et al.* 2004; Nielsen *et al.* 2006). Given the characteristic overdispersal of parasite populations in their hosts (Crofton 1971; Sréter *et al.* 1994; Galvani 2003) strongyle egg count data from mature horse populations are often dominated by individuals with zero or low egg counts. Since this tendency is consistent over time, some horses would appear to require fewer treatments than others in a prophylactic anthelmintic-based parasite control programme. This provides a basis for selective therapy, which has been promoted by several authors over the past 20 years (Duncan and Love 1991; Gomez and Georgi 1991; Krecek *et al.* 1994; Kaplan 2002; Matthee and McGeoch 2004).

It is generally accepted that faecal egg counts are more reliable during the grazing season. Studies in temperate climates have shown that encysted cyathostomins tend to accumulate during the autumn and winter months (Eysker *et al.* 1990). In addition, adult female worms appear to shed fewer eggs when it is not the grazing season (Poynter 1954). Taken together, this implies that samples taken during the grazing season are more reliable indicators of existing adult worm burdens, but there have been no studies to verify this.

Despite this discussion on the value of faecal egg counts in different situations, they play a vital role in equine parasite control as a means of detecting AR, through the use of the faecal egg count reduction test (FECRT) (Coles *et al.* 1992, 2006). This is considered the gold standard for detecting AR, and general

guidelines for the performance of this test are currently under development (see below).

Horse owners often express hesitation towards routinely taking faecal samples due to the costs associated with the analyses. Although the number of anthelmintic treatments often can be reduced with selective therapy, there can be substantial costs associated with the many faecal samples required on large farms. Some egg count kits have been made commercially available for simple and easy performance of the analyses (Presland *et al.* 2005), and with limited instruction, horse owners can reduce their expenses by performing the egg counts.

Larval cultures and identification of third stage larvae

Strongyle eggs are very similar in morphology and do not generally allow generic or specific differentiation (Hummelinck 1946). Although subtle differences among first (L₁) and second (L₂) stage larvae have been reported (Ogbourne 1971), identification cannot be based on examination of these stages. The third stage larvae (L₃), however, have more distinct morphological characteristics that allow several strongyle species to be identified (Russell 1948; Bevilaqua *et al.* 1993). Therefore, methods based on culturing of faeces and subsequent identification of L₃ have been used widely in research studies (Poynter 1954; Duncan 1974; Craven *et al.* 1998; Döpfer *et al.* 2004; von Samson-Himmelstjerna *et al.* 2007).

This procedure traditionally involves culturing jars of faeces for up to 2 weeks at room temperature, before larvae are harvested using a Baermann technique. Faeces can be mixed with an inert material like vermiculite to delay evaporation and facilitate oxygen availability in the centre of the faecal mass, but the consistency of equine faeces makes it an excellent culture medium even without vermiculite.

Strongyloides vulgaris L₃ are the most readily distinguishable of all strongyle third stage larvae, because of the high number (28–32) of distinct intestinal cells. Other species are not as easily identified, since several have 16–20 intestinal cells. Consequently, *S. edentatus* can only be distinguished from *Triodontophorus* spp. (*Strongylinae*), and *S. equinus* from *Poteriostomum* spp. (*Cyathostominae*), *Oesophagodontus robustus* (*Strongylinae*) and *Trichostrongylus axei* (*Trichostrongylidae*) by means of cell shape and other morphological characteristics (Russell 1948; Bevilaqua *et al.* 1993), and misclassifications can easily occur. Apart from *Poteriostomum* spp. (16 cells) cyathostomin species all have 8 intestinal cells and are easily distinguished from *Strongylus* species, but not from each other. Currently, *Gyalocephalus capitatus* (12 cells) has been placed outside the cyathostomin tribe, but can biologically still be considered a cyathostomin. Several reports have subdivided 8-cell cyathostomin larvae into a number of subgroups based on their morphometric analysis (Bevilaqua *et al.* 1993; Kornas *et al.* 2009). However, despite the potential for identification of individual strongyle genera, the single-most important reason for performing larval cultures in veterinary practice is to detect the presence of *Strongylus vulgaris*.

Although well described and often applied in the field, larval culture has never undergone a thorough validation as a diagnostic test for the detection of *S. vulgaris* in terms of sensitivity and specificity. As a means of large strongyle surveillance, this would be very useful information. With the development of molecular tools, there is the potential, in the future to be able to identify the species of adult strongyles present in a horse by rapid molecular analysis of the strongyle eggs present in faecal samples.

Baermann technique

Besides harvesting L₃ from larval cultures, the Baermann technique can also be used for the recovery of live parasite larvae or even adult worms in the faeces. For instance, the technique has been used to detect immature stages of cyathostomins in suspected cases of larval cyathostomosis (Olsen *et al.* 2003). Currently, this is the only way to detect the presence of an immature cyathostomin infection, but it is not widely used.

The Baermann technique is also considered the method of choice for detecting lungworm infection. However, it is of limited diagnostic value since *Dictyocaulus arnfieldi* is primarily a donkey parasite and normally very few worms reach sexual maturity in the horse. For the detection of lungworm larvae in their natural hosts, such as donkeys (*D. arnfieldi*), cattle (*D. viviparus*) and small ruminants (*D. filaria*, *Muellerius capillaris*), the Baermann technique is considered the simplest and most reliable diagnostic method. However, recent findings in small ruminants using the FLOTAC method for the detection of lungworm larvae indicated that this approach is more sensitive than the Baermann method and thus should be considered for this diagnosis also in horses (personal communication, Professor G. Cringoli, University of Naples, Italy).

Immunodiagnosis

Although there have been many attempts to develop immunodiagnostic assays over the past 20–30 years, very few have been validated for the diagnosis of equine helminth infections. Several assays have been developed for the detection of *Anoplocephala perfoliata* (Höglund *et al.* 1998; Proudman and Trees 1996), and the ELISA assay developed by Proudman and Trees (1996) has been made commercially available and has been used in several European countries. This assay measures serum antibodies against 12/13 kDa excretory/secretory (ES) antigens and has been validated in terms of diagnostic sensitivity and specificity as well as correlation with actual worm burdens. Generally, the assay has been found useful at a group level but the variability is too high for reliable diagnosis in individual horses (Proudman and Edwards, 1996; Morgan *et al.* 2005). This has been supported by a Danish abattoir study evaluating this assay, which found high background levels of antibodies in *A. perfoliata* negative horses as well as a high proportion of false positive samples (Kjær *et al.* 2007). This finding was supported by a study evaluating antibody levels after tapeworm treatment (Abbott *et al.* 2008), where horses were found to be antibody-positive for up to 5 months post treatment. In addition, recent data from horses coinfecting with *A. perfoliata* and *A. magna* suggest a lack of specificity of the serum antibody ELISA (Meana *et al.* 2009). Measuring antibodies has always led to difficulties in interpretation and has carried the inherent risk of overestimating infection prevalence. For example, the correlation between colic and *Anoplocephala* antibody levels is still a matter of controversy and recent investigations either suggest usefulness of the test for the assessment of the risk of colic (Boswinkel and Sloet van Oldruitenborgh-Oosterbaan 2007) or the opposite (Trotz-Williams *et al.* 2008).

Much effort has gone into developing serological assays targeting strongyle parasites. For instance, elevations of α - and β -globulin fractions and concurrent hypoalbuminaemia have frequently been associated with mixed strongyle infections (Schotman 1963; Drudge *et al.* 1966; Round 1971; Amborski *et al.*

1974; Duncan and Pirie 1975; Ooms *et al.* 1976; Schultze *et al.* 1983; Bailey *et al.* 1984; Thamsborg *et al.* 1998). However, most protein measurements were found to be nonspecific and subject to high degrees of variation with no particular pathognomonic pattern for strongyle infection (Bailey *et al.* 1984; Herd and Kent 1986; Abbott *et al.* 2007); to date there is no protein-based diagnostic assay for use in veterinary practice.

A major component of the increases in the globulin fraction in equine strongyle infections has been found to be due to increases in antibodies of the IgG(T) subgroup, which have been associated with *Strongylus vulgaris* infection (Patton *et al.* 1978; Wynne *et al.* 1981; Kent 1987). This finding led to the development of a commercially available assay termed Agglutinate Strongyle test¹, a latex agglutination kit measuring the level of IgG(T) in horse serum (Kent and Blackmore 1985a,b). Although an association with strongyle infection was observed, other causes of IgG(T) elevation could not be ruled out, since the specificity of antibodies was not evaluated. Thus, this assay was found to be of limited value on its own (Klei 1986), and it is no longer on the market. Instead, scientists attempted to develop specific serological assays detecting IgG(T) antibodies against *S. vulgaris* (Klei *et al.* 1983; Nichol and Masterson 1987; Weiland *et al.* 1991; Adeyefa 1992). Unfortunately, cross-reactivity with other nematode species seemed to constitute a major obstacle for development of a fully applicable diagnostic test and to date there is no satisfactory serological assay for the detection of *S. vulgaris* or any other equine strongyle parasite.

Molecular diagnosis

The diagnostic potential of gene sequences encoding ribosomes has been reported (Campbell *et al.* 1995; Gasser *et al.* 1996; Gasser and Monti 1997; Hung *et al.* 1999). This has led to several rDNA-based PCR assays detecting some important equine parasites.

A PCR assay been developed for the detection of *A. perfoliata* in faecal samples (Drögemüller *et al.* 2004), but the assay only performed slightly better than methods for detecting eggs based on the modified McMaster technique (Traversa *et al.* 2008).

A PCR-ELISA exploited the ribosomal intergenic spacer (IGS) region for identifying 6 species of cyathostomins, and has proven reliable and applicable for detecting the presence of these species in faecal samples (Hodgkinson *et al.* 2001, 2003, 2005). Similarly, Traversa *et al.* (2007) developed a reverse line blot assay also targeting the IGS region, which was capable of detecting 13 species of cyathostomins and all 3 species of *Strongylus*. Recently, the fluorescence-based real-time TaqMan PCR technology has been applied for the detection and semiquantification of DNA from *Strongylus vulgaris* in faecal samples (Nielsen *et al.* 2008).

Despite the promising results achieved with several of the above-mentioned molecular assays, none of them has yet been applied in the field. However, these studies may represent the first steps towards a molecular test panel capable of detecting all important parasites of horses in one analysis. This should be considered something worth striving for in future research projects.

Diagnostic tests of the future?

Larvae of cyathostomins can be highly pathogenic either when resident in the mucosa, because some stages are blood feeders, or when they emerge into the lumen and cause mucosal damage, which is the underlying pathogenesis of larval cyathostomosis

(Love *et al.* 1999). Since arrested mucosal L₃ and L₄ stages cannot be detected by coprology scientists have attempted to develop a tool for detecting encysted cyathostomins by testing for IgG(T) antibodies reacting with 2 antigens isolated from mucosal cyathostomins (Dowdall *et al.* 2002, 2003, 2004). This has shown great promise with positive correlations with encysted and luminal worm burdens, but no assay is as yet commercially available.

Coproantigen ELISAs have been developed for the detection of a number of helminth parasites. A coproantigen capture ELISA detecting secretory/excretory antigens from the nematode *Heligmosomoides polygyrus* in mice has shown promise for development of assays detecting gastrointestinal parasite burdens (Johnson *et al.* 1996). Since then, coproantigen capture ELISAs have been proposed for the detection of trematodes in cattle (Abdel-Rahman *et al.* 1999) and cestodes in dogs and humans (Allan *et al.* 1996; Deplazes *et al.* 1999). Recently there have been reports of a promising coproantigen ELISA detecting the equine tapeworm *Anoplocephala perfoliata* (Kania and Reinemeyer 2005; Skotarek *et al.* 2009). Therefore, there appears to be a potential for also detecting equine strongyle infections using this technique, but it remains unclear whether such methods would be more sensitive or reliable than current techniques for detecting strongyle eggs in faecal samples.

Anthelmintic resistance

Definition

Three decades ago Prichard *et al.* (1980) defined AR as a situation where there is '... a greater frequency of individuals within a population able to tolerate doses of compound than in a normal population of the same species.' Furthermore, it was stated that to fulfil the definition for AR, this trait needs to be heritable. This definition was applied in the reference paper of the World Association for the Advancement for Veterinary Parasitology (WAAVP) on recommended methods for the detection of AR (Coles *et al.* 1992). Within these guidelines, different thresholds for detection of AR using the faecal egg count reduction test (FECRT) in various livestock host species were suggested. For horses, faecal egg count reduction (FECR) below 90% was considered indicative of resistance. Various mathematical approaches have been used in the past to calculate the FECR and most recently Dobson *et al.* (2009) readvocated the use of arithmetic means as the basis for the FECR calculation. The fact that the current guidelines do not differentiate between drug classes, is a matter of concern (Coles *et al.* 2006) and will probably be addressed in a new version currently in preparation (Sangster 2008). Also, it would be of advantage if future recommendations would give more precise instructions on how the test should be carried out.

Diagnosis of anthelmintic resistance in horses

The FECRT is the most frequently used test to investigate the prevalence of AR in horses. It can be applied for all drug classes and is relatively simple to run. However, it is time-consuming and labour intensive and will presumably only detect resistance where a considerable proportion of the resident worm population shows the resistant phenotype (Taylor and Hunt 1989). For the FECRT the examination of faecal samples from at least 6 horses at the time of treatment and approximately 14 days post treatment is recommended (Coles *et al.* 2006). Furthermore, it is extremely

important to ensure that each animal receives the correct dosage of anthelmintic. Due to time and costs involved, the FECRT is only rarely applied in routine veterinary practice. A more pragmatic approach for evaluating drug efficacy could therefore be to perform FECs 2 weeks post treatment. If eggs are found post treatment, there will be an incentive to perform standard FECRTs. When macrocyclic lactones were used for treatment, a post treatment FEC at 3 or 4 weeks post treatment may allow the detection of low level resistance, which may be present at immature stages only, or could be due to an inhibitory effect on egg laying in female worms without eliminating them.

The only currently available *in vitro* test for horse nematodes is the egg hatch inhibition test (EHT), which is suitable for testing susceptibility of cyathostomins against benzimidazoles. This test has recently been standardised for ruminant nematodes (von Samson-Himmelstjerna *et al.* 2009) and can be considered applicable in horses although no validation studies have been published for equine nematodes. No reliable *in vitro* or molecular based tests are currently available to detect resistance in other drug classes.

Prevalence levels of anthelmintic resistance

The widespread prevalence of resistance of cyathostomins to benzimidazole (BZ) type drugs has been documented in numerous recent studies (Tarigo-Martinie *et al.* 2001; Witzendorff *et al.* 2003; Kaplan *et al.* 2004; Wirtherle *et al.* 2004; Meier and Hertzberg 2005; Lind *et al.* 2007). Compared with North America where it is quite commonly identified, the prevalence of pyrantel (PYR) resistance has remained fairly low in many other parts of the world. Nevertheless, PYR resistance has been described in Norway (Ihler 1995), Denmark (Craven *et al.* 1998), the USA (Kaplan *et al.* 2004) and Sweden (Lind *et al.* 2007). Up to the present time, the efficacy of the macrocyclic lactone (ML) group of drugs against cyathostomin has remained almost unchanged despite their widespread use in horses over the past 20–30 years. However, since the expiry of the patent protection for ML drugs, such as ivermectin (IVM) and moxidectin (MOX), many generic products have recently entered the market resulting in a considerable increase in ML usage in horses (von Samson-Himmelstjerna *et al.* 2007).

As a result of this, parasitologists anticipate to see the emergence of ML resistance in cyathostomins (Sangster 1999a). Sangster (1999a) predicted that a reduction of the egg reappearance period (ERP), particularly in foals, would be the first sign of resistance in cyathostomins. Recent studies have reported reduced ERPs of less than 5 weeks in Germany (von Samson-Himmelstjerna *et al.* 2007), Brazil (Molento *et al.* 2008), UK (Dudenev *et al.* 2008) and USA (Lyons *et al.* 2008). This is a much shorter ERP than that described in several earlier publications where the IVM ERP in horses was found to be at least 9 weeks (Borgsteede *et al.* 1993; Boersema *et al.* 1996). Most recently, Lyons *et al.* (2009) performed a critical test study clearly illustrating that the shorter ERP was indeed due to apparent ivermectin resistance of the luminal L₄ stages.

A multinational study evaluating the efficacy of BZ, PYR, IVM and MOX in a total of 102 horse yards from Germany, Italy and the UK confirmed a high prevalence of BZ resistance in cyathostomins (Traversa *et al.* 2009). PYR resistance was detected on approximately a quarter of the farms, resistance against IVM was found in one Italian and 2 UK yards while no signs of MOX resistance were found when performing FEC at 2 weeks post

treatment. Interestingly, one of the 22 UK yards had a parasite population with apparent resistance to FBZ, PYR and IVM concurrently, representing the first indication of multiple drug resistance in cyathostomins.

Parascaris equorum is considered the dose limiting species for ML drugs. This relates mainly to a variable efficacy against intestinal larval stages observed in naturally infected foals (DiPietro *et al.* 1987; French *et al.* 1988), since actual dose titration data are not available. The potential development of drug resistance in *P. equorum* was suspected in recent publications from Canada (Hearn and Peregrine 2003; Slocombe *et al.* 2007), USA (Craig *et al.* 2007), Sweden (Lindgren *et al.* 2008), Denmark (Schougaard and Nielsen 2007), England (Stoneham and Coles 2006), Germany (von Samson-Himmelstjerna *et al.* 2007), Italy (Veronesi *et al.* 2009) and the Netherlands (Boersema *et al.* 2002). In one study, foals were experimentally infected with a purportedly resistant isolate of *P. equorum*, and half of them were blindly treated with ivermectin. Subsequent necropsies revealed total treatment failure of ivermectin (Kaplan *et al.* 2006).

Anthelmintic resistance in large strongyles has so far not been reported, presumably due to the high efficacy of modern anthelmintics against both adult and larval stages and the extremely long prepatent periods leading to much longer generation intervals.

With the arrival of emodepside (Harder and von Samson-Himmelstjerna 2002) and monepantel (Kaminsky *et al.* 2008), 2 new mode of action nematocidal drugs have been introduced to the veterinary market and as such they are the first in more than 2 decades. A third compound, derquantel was recently presented, but it is not well tolerated by horses (Steven Maeder, Pfizer Ltd, personal communication). Thus, it remains unclear whether any of these compounds will be available for use in horses and equine parasitologists and practitioners would be well advised to design worm control programmes that will be sustainable using presently available drugs.

Clinical implications of anthelmintic resistance

Despite the widespread and long standing prevalence of BZ resistance in cyathostomins and the more recent discovery of ML resistance in *P. equorum*, to date the authors are unaware of any published cases of disease associated with AR. In the case of the cyathostomins this may be related to the fact that, with the exception of larval cyathostominosis, this group of parasites does not usually cause severe or specific disease symptoms. Infections with *P. equorum*, however, represent a much more severe threat to the health of foals and young horses. A first report about *P. equorum* caused death in a foal, suggesting that an association with ML-resistance recently appeared (Stoneham and Coles 2006) and it will presumably only be a matter of time until further reports of disease despite recent ML treatment.

Management of anthelmintic resistance

It has been shown that there is a direct relationship between the frequency of treatment and the rate of development of AR (Kelly *et al.* 1981; Uhlinger 1991; Herd 1993; Herd and Coles 1995). Therefore, a high frequency prophylactic dosing strategy, while effective in the short term, is not sustainable owing to the likely development of AR (Matthee *et al.* 2002). Selection pressure for AR is increased by treatment intervals equal to or shorter than either the parasite's prepatent period or the ERP following specific

drug treatment (Waller 1987). In such cases, susceptible worm populations do not reach patency in the short period between treatments, which results in the next parasite generation being composed mainly of resistant individuals. In addition, the development of an effective acquired immunity in young horses may be compromised (Herd 1990, 1993; Herd and Gabel 1990; Monahan *et al.* 1997).

Separate management programmes for different age groups are useful to limit the overall number of anthelmintic treatments used (Matthee *et al.* 2002), since young horses require more frequent treatment than adult animals (Herd and Gabel 1990; Little *et al.* 2003; Love 2003; Matthee and McGeoch 2004).

Drug rotations

The potential effect of drug class rotation on development of AR has been evaluated using computer model calculations for sheep trichostrongyles. With this approach, there was no apparent advantage of either rotation with each treatment, or annual, 5 or 10 year rotations (Barnes *et al.* 1995). Similarly, for cyathostomins a field study showed that rotating between drug classes with every treatment did not appear to slow development of AR (Uhlinger and Kristula 1992). On the contrary, it is speculated that drug class rotation may even increase the rate at which resistance develops by selecting for resistance to more than one drug simultaneously (Kaplan 2002). When more than one anthelmintic class has broad spectrum activity against the most important parasites, annual rotation has been recommended (Herd and Coles 1995). However, because available anthelmintics lack activity against all types of parasites, specific treatments for parasites such as bots and tapeworms may be required. Annual drug class rotation can contribute to a sustainable worm control by reducing the selection pressure to the individual drug classes. However, it first needs to be established which drug classes are still active on the individual farm. A practical and meaningful approach could then be to alternate according to spectrum (e.g. use MLs in late autumn if a treatment for bots is required) or time of year (e.g. in spring and early summer use a drug class aimed primarily at the removal of adult worms thus reducing the subsequent potential for pasture contamination with infective eggs or larvae).

Use of incorrect dosage rates

Incorrect dosing is one of several factors that may facilitate the development of AR (Kelly *et al.* 1981; Wescott 1986; Waller 1987). The most often used approach to decide on dose rate is visual weight estimation, which is often inappropriate and can lead to underdosing. If the same dosage is used for treating individuals in a group of similar animals, it should be based on the weight of the heaviest animal in the group (Coles *et al.* 1992). An easy, inexpensive and reliable method for reasonably accurate weight estimation in horses is the use of a girth tape (Pook *et al.* 2002) but, according to recent surveys in the UK, Germany and Italy, this is rarely used in practice (von Samson-Himmelstjerna *et al.* 2009; Fritzen *et al.* 2010).

Off-label drug usage

On 11.8% of more than 70 German horse farms examined, it was found that doramectin (DOM, i.e. Dectomax) was or had been used (Fritzen 2005). This drug formulation is not labelled for equine use,

and a similar observation was made in South Africa by Matthee (2003). DOM is considerably cheaper than registered equine formulations, making it an attractive option for many horse owners. In addition, 13% of the German farms admitted to the use of ruminant formulations of injectable IVM. From these observations it can be assumed that off-label use of anthelmintic products is not uncommon in equine establishments. This is of great concern, since it cannot be assumed that the pharmacokinetics of these drug formulations in horses is similar to that in ruminants. Thus, off-label usage carries a substantial risk of a reduction in anthelmintic efficacy, which, in turn may potentially accelerate the development of AR. This has been supported by the findings of Matthee (2003) who observed therapeutic failure of injectable DOM and MOX used i.m. on South African stud farms with a history of off-label usage of these drugs.

Pasture management

Parasite refugia

For parasitic nematodes of small ruminants it was shown that the size of the parasite refugium can have a considerable effect on the development of AR (van Wyk 2001; Wolstenholme *et al.* 2004; Waghorn *et al.* 2008). The proportion of a parasite population that is not exposed to the drug at the time of treatment is considered in refugium. Accordingly, free-living stages on pasture constitute a major part of the refugium, but also included are parasites in untreated individuals and parasitic stages not coming into contact with the drug, such as encysted cyathostomins when treated with non larvicidal anthelmintics (Nielsen *et al.* 2007). Accordingly, treatments directed also at the elimination of encysted larval stages of cyathostomins such as a 5 day fenbendazole course of treatment or the use of moxidectin may have a stronger resistance selecting effect.

Parasites in refugia are not exposed to selection for AR, and thereby provide a source of susceptible alleles in the population (Sangster 1999a; van Wyk 2001). An adequate parasite refugium can contribute to a reduced potential for the development of AR as was found in experimental studies in sheep (Martin *et al.* 1981; Dobson *et al.* 2001; Waghorn *et al.* 2008) and was suggested by computer modelling (Barnes and Dobson 1990; Smith *et al.* 1999).

As mentioned above, several studies have suggested selective therapy or targeted selective treatments (TST), i.e. treatment is only administered to animals with faecal egg excretion above a certain threshold or to animals in poor body condition. This may be a practical approach to maintain parasite refugium and AR management in adult animals (Duncan and Love 1991; Krecek *et al.* 1994; Little *et al.* 2003; Matthee and McGeoch 2004). Selective treatment, however, is generally not recommended for yearlings or foals (Matthee and McGeoch 2004). The FEC threshold used to select animals for treatment may vary between age groups, farms and regions. Ideally the chosen infection threshold must be low enough to prevent clinical disease associated with helminth infection, but high enough to ensure that a portion of the worm population within the herd will be left untreated and contribute to the parasite population in refugium (Matthee and McGeoch 2004). However, as already discussed, the number of parasite eggs shed in the faeces does not necessarily correspond with the number of parasites present in the intestine, and this could have significant implications on the use of TST approaches alone to control strongyle infections, in horses.

Since low or negative strongyle FECs do not necessarily indicate low or negative worm burdens, the advice not to treat an individual horse bears certain potential risks. For example, a high burden of immature parasites may be present, which, if left untreated, could continue their development and either result in clinical disease or significant pasture contamination. In equine establishments, treatment decisions are often made for individual animals and not on a herd basis. In riding stables or livery yards, for example, there may be many owners using different anthelmintics in different dosing regimes on the advice of several independent veterinarians.

On the other hand, if an overall parasite control programme is in place and under veterinary supervision, it may be impossible to exclude from treatment horses with low or negative FECs. If the primary goal of anthelmintic treatments is to reduce pasture contamination, however, recent investigations have suggested that FECs can be a reliable indicator of the requirement for and treatment frequency of individual animals, due to the fact that many adult horses tend to maintain consistently low FECs (Gomez and Georgi 1991; Döpfer *et al.* 2004; Matthee and McGeoch 2004; Nielsen *et al.* 2006).

A practical problem if using TST as a basis for parasite control is the costs of faecal egg count analysis. Currently, the laboratory costs of faecal examination usually exceed the costs of treating a horse. This means that horse owners will often be reluctant to have faecal examinations performed. Costs could be greatly reduced when and if it is established which horses are consistent high egg shedders and therefore should be wormed frequently and which horses consistently have negative counts or shed low numbers of eggs in the faeces. A possible reduction in costs may be obtained by using composite or group samples from animals of the same age group (i.e. foals, yearlings or adults) and initial studies indicate that this could be appropriate (Eysker *et al.* 2008).

Dose and move strategies

It has been shown that dose and move strategies to reduce contamination of pastures after anthelmintic treatment can be effective in reducing levels of infection (Ribbeck *et al.* 1997; Lyons *et al.* 1999). However, although frequently recommended in the past, use of such techniques is now more controversial since it is considered to increase the selection for AR through reducing the proportion of susceptible parasites in refugium on the new pasture (van Wyk 2001; Matthee *et al.* 2002). At present it is not known for various horse management systems, what level of contamination is acceptable without leading to clinical disease on the one hand and on the other hand what level is required to maintain enough susceptible worms to delay development of resistance (Coles 2005) or to allow the establishment of an effective acquired immunity.

Removal of faeces or harrowing, ploughing and reseeding of pastures

Previous studies (Duncan 1985; Herd 1986a,b; Herd and Coles 1995) reported a reduced prevalence of strongyle infection in herds kept on pasture where faeces were removed regularly. It markedly reduces the free-living stages and thereby the number of anthelmintic treatments necessary (Lloyd *et al.* 2000). Faecal material should be removed at least once weekly for this to be effective.

Following deep ploughing and reseeded the pasture would be expected to be free of strongyle larvae, although data from pig farms on the same approach used for the control of ascarid infections suggest that *P. equorum* eggs may not be affected as much (Mejer 2006). However, it should be borne in mind that the grazing behaviour of pigs is considerably different from horses with the pigs digging deep into the soil.

Stable and pasture hygiene has been found to influence levels of parasite infections. For example, fertilising pastures with horse manure can lead to a higher prevalence of *P. equorum* (Lyons *et al.* 1999; Fritzen *et al.* 2010), while good stable hygiene achieved by daily cleaning and regular disinfection of stalls has been associated with a reduced ascarid and strongyle prevalence (Fritzen 2005; Fritzen *et al.* 2010). In practice, good stable hygiene is more important with regard to control of ascarid infections as strongyle infections mainly occur at pasture (Reinemeyer 1986, 1998).

Stabling horses after anthelmintic treatment

The potential risk of contamination by stages of worms expelled following anthelmintic treatment should be considered. This is probably of greater relevance in the case of *P. equorum* than in other equine helminth parasites. It is unlikely that a significant number of strongyle eggs present in expelled females will hatch or result in the development of free living third stage larvae. In contrast, there is the possibility that fertilised *P. equorum* eggs may persist in the environment and may potentially develop through to the infective stage following destruction of the dead female worm. Although, no experimental data exist to support this assumption, it is probably advisable to keep *P. equorum* infected animals indoors for 3 days post treatment and to ensure that the litter produced during this period is not used to fertilise horse pastures.

Conclusions

The present review provides current opinions on practical equine parasite control. A large body of evidence now exists confirming the widespread occurrence of AR in equine establishments world wide, and this warrants an increased focus on all aspects of parasite control. Due to this changing situation, new questions have arisen and the purpose of the AVEF workshop was to attempt to answer some of these.

Traditional diagnostic tools used in equine parasitology are well described, but have never been rigorously evaluated in terms of diagnostic sensitivity and specificity. Given the increasing emphasis on the use of faecal egg counts in parasite control programmes, there is an increased requirement for such evaluations. Immunodiagnosics have not been shown to be useful in equine parasitology, and although molecular tools have been developed recently, no molecular based test is yet commercially available.

For decades the main goal of anthelmintic intervention was the prevention of parasitic disease and the optimisation of equine health. With the current status of AR it is clear that it has become just as important to maintain efficacy of existing drug formulations for as long as possible. Therefore, equine parasite control has become a matter of finding the balance in terms of anthelmintic usage. At present we have very little evidence of the long-term consequences of various suggested treatment approaches, but it has

become increasingly clear that treating all horses at fixed intervals all year round can no longer be considered a valid approach. Factors such as age, physiological status, function (sports, leisure or breeding), and farming and management practices should be considered when designing any equine parasite control programme. Routine testing for anthelmintic resistance using the faecal egg count reduction method should also be implemented on all equine establishments.

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Manufacturers' addresses

¹Virbac, Carros cedex, France.

Further reading

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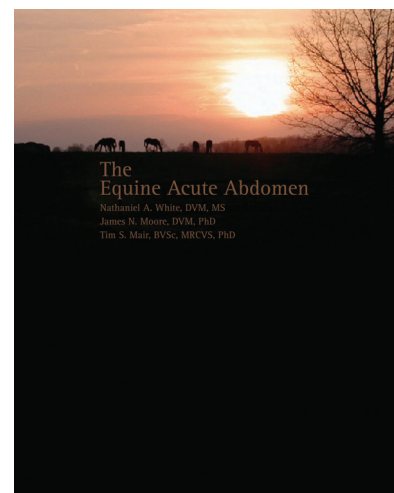
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