

# AAEP Parasite Control Guidelines

## Mission Statement

Commonly used strategies for parasite control in adult horses are based largely on knowledge and concepts that are more than 50 years old. However, much has changed over this time and information presented on current product labels represents historic information about anti-parasitic efficacy but does not necessarily reflect current product performance. This development necessitates a re-examination of recommendations for parasite control. In response to this need, the AAEP formed a Task Force charged with producing a comprehensive set of recommendations for helping veterinarians develop improved strategies and programs for parasite control in horses of all ages. Guidelines will be specified separately for adult and young horses (less than 3 years).

Recommendations developed in this document are based on the following:

1. Important changes in the parasitic fauna of horses have occurred such that *Strongylus vulgaris* and other large strongyles are now rare, and cyathostomins (small strongyles) and tapeworms are now the major parasites of concern in adult horses, while *Parascaris* spp. remains the most important parasite infecting foals and weanlings.
2. Anthelmintic resistance is highly prevalent in cyathostomins and *Parascaris* spp., and this must be factored into treatment decisions (Kaplan and Nielsen, 2010).
3. Adult horses vary greatly in their innate susceptibility to infection with cyathostomins and their level of strongyle egg shedding and thus, require individualized attention to their parasite control needs.
4. Horses less than about 3 years of age require special attention as they are more susceptible to parasite infection, and are more at risk for developing disease. This article will detail the separate approach taken for parasite control in this age group.

## AAEP Parasite Control Guidelines

### Introduction

Traditional parasite control programs involving rotational treatment with anthelmintics at regular intervals are commonly recommended by veterinarians. However, this approach is based on concepts and strategies developed more than 50 years ago when *Strongylus vulgaris* (large strongyle bloodworm) was the most important parasitic pathogen of horses (Drudge and Lyons, 1966). The rationale for this parasite control scheme was rather simple: to kill *S. vulgaris* worms before they could mature and lay eggs that would contaminate the environment. Since it took about two months for strongyle eggs to reappear after treatment, treatment every two months prevented *S. vulgaris* eggs from being shed on pastures. This approach was very successful in controlling *S. vulgaris* infections, and disease from *S. vulgaris* is now very rare in managed horse populations.

It is noteworthy that cyathostomins (small strongyles), were not considered important pathogens at that time, as their pathogenic potential was over-shadowed by *S. vulgaris*. However, that situation has changed and currently, cyathostomins (small strongyles), are recognized as a primary equine parasite pathogen (Love et al., 1999). Similarly, *Parascaris* spp. is recognized as a major parasitic pathogen in foals and weanlings (Nielsen, 2016a), and *Anoplocephala perfoliata* has been recognized as a cause of ileal colic in the horse (Nielsen, 2016b). The biology, life-cycles and host-parasite dynamics of the cyathostomins, *A. perfoliata* and *Parascaris* spp. are very different from *S. vulgaris*, thus strategies designed for controlling *S. vulgaris* will not be appropriate or very effective for controlling these parasites.

Decades of frequent anthelmintic use have selected for high levels of anthelmintic drug resistance in cyathostomin and *Parascaris* spp. populations (Peregrine et al., 2014), which emphasizes that the traditional approaches for parasite control are not sustainable and that new strategies are needed.

Cyathostomins are truly ubiquitous, and all grazing horses are infected. But they are relatively mild pathogens and only produce disease when infections reach extremely high levels. Thus disease from strongyle parasites is much less of a concern in adult horses today than it was decades ago when *S. vulgaris* was highly prevalent. Frequent anthelmintic treatments are therefore not needed to keep adult horses healthy. What is needed are properly timed treatments with effective anthelmintics administered at the appropriate time of the year, which correspond to the epidemiological cycles of transmission and the relative parasite burdens in individual horses. In this document we aim to provide the information necessary to implement parasite control programs for adult horses based on the best available evidence.

## **TERMINOLOGY TO KNOW AND UNDERSTAND**

There are definitions and terminology that are used by parasitologists when discussing equine parasitology. Commonly used terms have been included to assist in developing a common verbiage for both the veterinarian and horse owner.

### **ANTHELMINTIC RESISTANCE**

“Resistance is the ability of worms in a population to survive treatments that are generally effective against the same species and stage of infection. Anthelmintic resistance is an inherited trait. The development of resistance first requires that resistance genes are present. The rate of development of resistance is determined by selection pressure and the extent to which worms surviving treatment pass their genes on to the next generation. With continued selection and reproduction of resistant worms, the frequency of resistance genes in the local worm population increases to the point where treatment fails. Once resistance is present, the population of resistant parasites do not appear to revert to susceptibility, so the aims of resistance control are to prevent the first steps in the development of resistance and then to delay the accumulation of resistance genes.” (Sangster, 1999)

- Anthelmintics (dewormers) select for parasites in the population that have mutations that confer drug resistance to that drug. Repeated anthelmintic treatments allow the resistant parasites to preferentially survive and increase in frequency over time.
- The Fecal Egg Count Reduction Test (FECRT) is the only method currently available for detecting resistance in parasites of horses.

Current levels of anthelmintic resistance in equine parasites are summarized in table 1. The occurrence of resistance is very variable and large differences can be found between individual farms, and resistance cannot be concluded on any given farm without proper testing. Thus, table 1 only presents which parasites are most likely to show resistance to which drug class.

**Table 1.** Current levels of resistance documented in peer-reviewed studies in major nematode parasites to the three anthelmintic classes in managed horse herds. These are world-wide trends that have also been reported in several US surveys.

Drug class	Cyathostomins	Large strongyles	<i>Parascaris</i> spp.
Benzimidazoles	Widespread	None	Early indications
Pyrimidines	Common	None	Early indications
Macrocyclic lactones	Early indications	None	Widespread

*Widespread:* reported on multiple continents with high farm prevalences often above 80%

*Common:* reported on multiple continents with varying farm prevalences

*Early indications:* few single farm cases of reduced efficacy (ascarids) or reports of reduced egg reappearance periods (strongyles)

## PARASITE REFUGIA

Refugia refer to the portion of a population of parasites (or stages of parasites) that eludes the drug at the time of a treatment event. This sub-population includes stages of parasites in the horse not affected by the treatment (e.g., encysted cyathostomins when non-larvicidal treatments are used), all free-living parasite stages on the pasture, and all parasites in animals that were not treated. The higher the proportion of worms in refugia, the more slowly resistance develops. The worms in refugia are not under selection pressure for resistance, thus resistant worms remain diluted by susceptible worms, which continue to make up the majority of the worm population (Leathwick et al., 2008; Waghorn et al., 2008).

Examples of anthelmintic formulations that do not treat all parasitic stages within the horse include pyrantel formulations that have no efficacy against parasite stages present outside the gastrointestinal lumen, and ivermectin which has no documented efficacy against encysted cyathostomin larvae.

The concept of refugia can be utilized by keeping the frequency of drug treatments at a minimum when pasture refugia is low (e.g., during the temperature extremes of cold winters or hot summers and during droughts). Consequently, the old practice of “dose-and-move”, is now considered to select more strongly for resistance, as moving newly dewormed horses to a new pasture removes the dilution effect that would have been provided by a good size pasture refugia (Waghorn et al., 2009).

Furthermore, refugia can be utilized by leaving some horses untreated at every deworming. Fecal egg counts can be used to select the moderate and high egg shedders for anthelmintic treatment. One study illustrated that if highly effective drugs are used, treating all adult horses exceeding a strongyle FEC of 200 EPG, only leads to treating about 50% of the horse population, but still provides about 95% reduction of the overall egg shedding (Kaplan and Nielsen, 2010).

### **FECAL EGG COUNT REDUCTION TEST (FECRT)**

The FECRT is used to determine if strongyles and/or ascarids are resistant to a given anthelmintic. However, a finding of reduced efficacy may or may not mean there is resistance present. Therefore, suggested cutoffs should be viewed as a guide for interpretation, but not be viewed as the final answer. To perform the FECRT a fecal sample is collected prior to deworming. The anthelmintic in question is administered and a fecal sample is collected 10-14 days following treatment. Using the equation below, the number of eggs in the pre-treatment and post-treatment fecal samples is used to calculate the percent reduction in FEC for a group of horses. The mean reduction for all horses tested is calculated to determine the percent reduction for the farm or stable. This value is then used to make inferences regarding possible presence or absence of drug resistance.

$$\frac{\text{EPG (pre-treatment)} - \text{EPG (14 day post-treatment)}}{\text{EPG (pre-treatment)}} \times 100 = \text{FECRT}$$

Specific guidelines for FECRT in horses do not currently exist, but are being developed by parasitologists under the auspices of the World Association for the Advancement of Veterinary Parasitology (WAAVP). Until those guidelines are published, the cutoff values listed in Table 2 should be used as a guide for interpreting the results of a FECRT.

**Table 2:** Suggested cutoff values (mean percent reduction in FEC) for interpreting results of strongyle FECRT

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

\* As of March 2019, full-fledged strongyle resistance as diagnosed with a standard FECRT to ivermectin or moxidectin has not been reported in the peer-reviewed literature. Therefore, any FECRT result that yields <95% reduction for these drugs should be repeated before concluding there is resistance.

It is recommended to include at least six egg count positive horses in a FECRT on each farm to evaluate treatment efficacy. Furthermore, it is recommended to always recruit the horses with the highest possible pre-treatment egg count for the FECRT, and to use an egg counting technique with a limit for detection of less than 25 EPG (see Appendix A). Horses should not have received anthelmintic treatment at least 8 weeks prior to the FECRT, and should preferably have been residents on the given farm for at least one year for their parasites to be representative of the general parasitic fauna on the farm. When interpreting results of a FECRT it is important to appreciate that there are many factors that can affect the observed results of a FECRT (see Vidyashankar et al., 2012 for details). FEC are by their very nature quite variable, so if testing is done with few horses there is potential for high variability, which could lead to an incorrect inference. An FECRT can be carried out with fewer than six horses, but the results should be interpreted with caution. In case of borderline results or cases of reduced efficacy indicated only in some, but not all horses, the test should be repeated before any firm conclusion is made.

In addition, all horses sharing pastures share the same population of parasites, and resistance should always be evident across that population. It is not biologically possible that resistant worms are present in some horses but not others. However, unless efficacy is very high for all horses tested, high variability in results among the horses is quite common. Ultimately, FECRT results can only be interpreted for the population (herd) and not on the individual level. It should always be borne in mind that a borderline reduced efficacy can be caused by factors other than resistance, such as incorrect dosage, incorrect storage, and expired product used.

### **EGG REAPPEARANCE PERIOD (ERP)**

The ERP is defined as the time interval between the last effective anthelmintic treatment and the resumption of strongyle egg shedding. Leading equine parasitologists in the US suggest the following definition be used: the post-treatment week when the percent reduction in FEC decreases below a cutoff value of 80% for benzimidazoles and pyrantel, and below 90% efficacy for ivermectin and moxidectin. This is measured by performing weekly FECRTs until egg reappearance is seen. The ERP is irrelevant if drug resistance to a particular anthelmintic is already present on a given property, as there is no egg disappearance. Monitoring ERP on a

farm over time has value because a shortening of the ERP is a precursor to the development of resistance. Monitoring ERPs has the most practical implication for measuring possible emergence of resistance to ivermectin and moxidectin. Table 3 shows the usual ERP for the common equine anthelmintics, when they are fully effective at two weeks post treatment, as well as recently reported ranges of shortened ERPs. Macrocyclic lactones are characterized by very long ERPs, but recent reports have documented them shortened to just 4-5 weeks for both ivermectin and moxidectin on managed farms (Lyons et al., 2008a; Rossano et al., 2010; Lyons et al., 2011; Bellaw et al., 2018). This is interpreted as emerging resistance in cyathostomins to this drug class (Sangster, 1999). Thus, for macrocyclic lactones it can be of value to run one set of post-treatment egg counts at approximately 4-6 weeks to gauge the ERP status on a given farm.

**Table 3:** Cyathostomin egg reappearance periods (ERP) for equine anthelmintics

<b>Anthelmintic</b>	<b>Usual ERP when drug is effective</b>	<b>ERP when drug was first introduced</b>	<b>ERPs on farms with emerging resistance</b>
Fenbendazole/Oxibendazole	4-5 weeks	6 weeks <sup>a</sup>	- <sup>e</sup>
Pyrantel	4-5 weeks	5-6 weeks <sup>b</sup>	- <sup>e</sup>
Ivermectin	6-8 weeks	9-13 weeks <sup>c</sup>	3-5 weeks
Moxidectin	10-12 weeks	16-22 weeks <sup>d</sup>	4-6 weeks

<sup>a</sup>McBeath et al., 1978.

<sup>b</sup>Boersema et al., 1995; 1996.

<sup>c</sup>Borgsteede et al., 1993, Boersema et al., 1996, Demeulenaere et al., 1997.

<sup>d</sup>Jacobs et al., 1995, DiPietro et al., 1997, Demeulenaere et al., 1997.

<sup>e</sup> Resistance so commonly reported that ERPs have not been measured.

### **STRONGYLE EGG SHEDDING/CONTAMINATION POTENTIAL**

Although horses grazing together share the same parasite population, they demonstrate huge differences in their levels of strongyle egg shedding. Within any group of mature horses (> 3 yrs. of age), strongyle egg counts are highly concentrated in certain horses, such that 15 – 30% of adult horses usually shed approximately 80% of the eggs (Kaplan and Nielsen, 2010). This distribution of parasite egg shedding among hosts is common to all species and is referred to as over-dispersion. This characteristic for a horse is very stable over time, when it is otherwise in good health, pasture management practices are sound, and the horse has not recently moved from one farm to another. Thus, a healthy pastured horse with a low egg shedding potential will tend to always have a low FEC, while a healthy pastured horse with a high egg shedding potential will tend to always have a high FEC (Nielsen et al., 2006; Becher et al., 2010).

In order to determine the egg shedding potential for an individual horse, it is necessary to collect a fecal sample and perform a fecal egg count (FEC) after the effects of the last dewormer administered are completely gone. If you do not wait a suitable period of time following the last anthelmintic treatment, the results of the FEC will only reflect the efficacy of the last dewormer used, rather than measuring the innate ability of the horse's immune system to regulate levels of cyathostomin egg shedding. Older studies have illustrated that parasites reduce their egg

shedding outside the grazing season, where conditions are less favorable for parasite transmission (Poynter, 1954), but this pattern may well depend on geographical and climatic conditions and it is not certain to which extent this is a global phenomenon.

To evaluate the egg shedding status in adult horses (> 3 yrs. of age), a fecal sample should be collected a minimum of 4 weeks beyond the Egg Reappearance Period (ERP) for the last drug used. Given the information in Table 3, a washout period of 8 weeks should suffice in the large majority of cases, but it can be extended for macrocyclic lactones in cases where ERPs are not reduced to the 4-5 week range.

There are little data available for scientifically setting the FEC thresholds used for dividing adult horses into low, moderate and high categories for egg shedding. However, one study reported that strongyle FEC cutoff values up to the level of 500 EPG yielded significantly different strongyle worm counts, whereas no differences were found at higher cutoffs. These data therefore support usage of cutoffs for treatment in the 0-500 EPG range (Nielsen et al., 2010a). Nonetheless, currently recommended thresholds are based largely on the opinions of a majority of equine parasitologists, and as such could change as more data are collected and analyzed. Guidelines for classifying horses on the basis of egg contamination potential are presented in table 4.

**Table 4.** Suggested guidelines for classifying horses into different levels of strongyle egg shedding and the expected percentage of the horse population belonging to each group (Kaplan and Nielsen, 2010).

Egg count level		Percentage of adult population <sup>a</sup>
Low contaminators:	0-200 EPG	50-75
Moderate contaminators:	200-500 EPG	5-15
High contaminators:	>500 EPG	10-30

<sup>a</sup> These values are only estimates and the actual percentage of horses in each category will vary among farms depending on a multitude of factors

It is generally advised to classify adult horses to the three strongyle contaminative groups based on more than just one egg count performed at one point in time. In a Danish study where FEC were performed every six months over three years, greater than 90% of horses with FEC < 200 EPG on two consecutive fecal exams had a FEC of less than 200 EPG on the third (Nielsen et al., 2006). Thus, it appears that egg shedding categories for most horses remain consistent, but some horses may switch categories, particularly those with FEC near the cutoff values.

## GOALS OF PARASITE CONTROL

The true goal of parasite control in horses (and other equids) is to limit parasite infections so animals remain healthy and clinical illness does not develop. The goal is NOT to eradicate all parasites from a particular individual. Not only is eradication impossible to achieve, the inevitable result is accelerated development of parasite drug resistance. In addition, both small and large strongyles cause the greatest disease during their larval stages, which are refractory to most anthelmintic treatments. Consequently, most treatments that kill only adult strongyle

worms yield limited direct benefit to the horse. However, treatments effective against adult stages have an indirect benefit in that they prevent further contamination of the environment with infective stages. The resulting corollary is that to achieve good parasite control, one must prevent contamination of the living environment of a horse or horses with high numbers of parasite eggs and larvae. Thus, treatments should be timed to control the level of egg shedding into the environment. This relies on the use of deworming medications that are effective for their intended use. But treatments are only necessary when the environmental conditions are conducive to egg and larval development and survival. If strongyle eggs and developing larvae will be rapidly killed by the adverse environmental conditions (such as hot summers) or temperatures are too low to allow egg hatching and larval development to the third infective stage (such as cold winters) (Nielsen et al., 2007), then little is gained by deworming the horse if the horse is not showing any clinical symptoms of parasitic disease.

The goal of any parasite control program can therefore be summarized as follows:

1. To minimize the risk of parasitic disease.
2. To control parasite egg shedding.
3. To maintain efficacious drugs and avoid further development of anthelmintic resistance as much as possible.

To achieve these goals, it is important to know the magnitude of egg shedding of individual horses. This information can only be generated by performing periodic FEC surveillance. As noted above, the acceptable limits of strongyle EPG for a horse remain debated, and the egg shedding status of a horse may change over time as a result of changes in the horse's immune status and level of parasite exposure. In addition, no exact guidelines have been published regarding the "acceptable" number of *Parascaris* spp. eggs in young horses. However, even with these limitations in our knowledge, the magnitude of the FEC is the only means available to estimate the worm burden and egg contamination potential of a horse, and determine the effectiveness of anthelmintics. Consequently performing FEC surveillance is necessary to properly develop and monitor any parasite control program.

### **FECAL SAMPLING AND FECAL EXAMINATION**

There is a large number of techniques available for generating fecal egg counts in equines, and Appendix A provides protocols for two of the most widely used techniques. Automated smartphone-based egg counting systems are currently under development and will be made commercially available to veterinarians in the near future.

### **REASONS TO PERFORM FECAL EGG COUNTS (FEC)**

- To evaluate the anthelmintic efficacy using the FECRT.
- To evaluate and monitor the strongyle egg reappearance period (ERP) of the most recently administered dewormer.
- To determine the shedding status of the adult horse at the time of sampling.
- To determine whether parasite burdens in foals and weanlings are primarily *Parascaris* spp. or strongyle.

### **LIMITATIONS OF FEC**



- They do not accurately reflect the total adult strongyle or *Parascaris* spp. burden of the horse.
- They do not detect immature or larval stages of parasites including migrating large strongyles and ascarids, and/or encysted cyathostomins.
- Tapeworm infections are often missed or underestimated by fecal float techniques, and modified techniques are required.
- Pinworm eggs are usually missed since they are adhered as egg packets around the anus rather than being shed in the feces.

## **RECOMMENDATIONS FOR FECAL SAMPLING AND STORAGE**

- Samples should be stored in airtight and leak-proof containers or plastic bags.
- Collected manure should be as fresh as possible. Samples less than 12 hours old are acceptable, but should be refrigerated immediately after collection (Nielsen et al., 2010b).
- Refrigeration is always recommended for storage of fecal samples, but anaerobic storage at room temperature will also prevent eggs from hatching. Anaerobic storage can be achieved by squeezing all the air out of the bag, or by using a vacuum-sealing device. Note that anaerobic storage works best on wet feces; if feces are dry, it is difficult to achieve an anaerobic state.
- Samples should preferably be tested within 7 days of collection, although there are indications that eggs can remain intact for longer if adequately refrigerated.
- Fecal samples that are or have been frozen are not acceptable, as this will damage the eggs and decrease the recovery rate.
- Diarrhea samples are not acceptable for FEC, but can be used for qualitative testing. Note that if a horse has diarrhea that may be associated with parasitism, deworming may be indicated per clinician's recommendations without regard to results of the FEC.

## **FEC Training and Microscope Maintenance**

- Make sure that microscope lenses are adjusted to the working distance offered by parasitology slides used for the egg counts.
- Make good use of contrast (aperture condenser) to get a better image of morphological features.
- To improve skills at parasite egg identification, several resources are available online and through use of textbooks. One should consider review by a veterinary parasitologist if questions arise.
- It is recommended that microscopes be equipped with an ocular micrometer so that eggs and other questionable objects can be measured. Having measurements can greatly assist in the identification. Cheap digital cameras can now be acquired with software allowing these measurements.

## **INTERPRETATION OF EGG COUNT DATA**

In managed horses, greater than 99% of all strongyle eggs seen in a fecal sample are from the cyathostomins. In feral horses or in cases of severe neglect, 90-99% of the eggs seen will be from the cyathostomins and the remaining few percent will be from several large strongyle species, which are potentially more pathogenic. It is not possible to distinguish a large strongyle egg from a small strongyle egg while doing a FEC. Identification of large strongyle parasites requires culturing the feces and recovering and identifying the L3 larvae. This procedure is not difficult to learn but does require training. Arval culture and ID procedure presently is not offered by commercial laboratories but may be available in a few university veterinary diagnostic

laboratories. An ELISA test recently has been developed to detect the presence of *Strongylus vulgaris* larvae in the bloodstream (Andersen et al., 2013). This is currently commercially available in Europe and is offered by the Nielsen Laboratory at the University of Kentucky in the USA.

## **"OTHER" GASTROINTESTINAL PARASITES**

### ***Anoplocephala perfoliata* (Tapeworms)**

Necropsy surveys performed in Kentucky reported prevalences for *Anoplocephala perfoliata* of approximately 50% both prior to and after the introduction of widespread use of cestocidal drugs in horses (Lyons et al., 1984; Lyons et al., 2000; Lyons et al., 2018). The prevalence in other areas is unknown; however in much of the US tapeworms remain common. Oribatid mites serve as the intermediate host for tapeworms, and are commonly found on grass pastures. Though epidemiological data are limited, it appears that higher densities of oribatid mites occur where moist environmental conditions are found and in arid areas few or low numbers of oribatid mites are present leading to a lower incidence of tapeworm infection.

In recent years, parasite *A. perfoliata* has received growing attention as a potential pathogen causing various types of colic (Proudman et al., 1998; Nielsen, 2016b). Several studies have found an association between presence of tapeworms and colic originating from the ileocecal region (Nielsen, 2016b). Tapeworms produce small mucosal erosions at the site of attachment and when present in relatively high numbers, have been associated with ileocecal impactions and spasmodic colic (Proudman et al., 1998). However, most horses infected with tapeworms tend to have relatively few worms, and these likely produce little in the way of pathogenic consequences.

The diagnostic sensitivity of the general McMaster technique for diagnosing equine tapeworm infections is less than 10% (Nielsen, 2016b). Consequently, unless a horse is infected with a large burden of tapeworms, seeing tapeworm eggs in the feces is a chance event, when a standard egg counting method is used. A modification of a centrifugation-based egg counting technique based on analyzing 40 grams of feces has been validated to have a diagnostic sensitivity and specificity of 0.61 and 0.98, respectively (Proudman and Edwards, 1992). For detecting tapeworm burdens of 20 worms and above, the sensitivity of this method was found to be 0.90, which is very good for a parasitological diagnostic test. One study has found a Wisconsin method set to analyze 5 grams of feces to have a diagnostic sensitivity of 0.62 (Slocombe, 2004). The number of eggs seen is not highly relevant, and fecals should just be interpreted as being either negative or positive. To greatly increase the sensitivity of detection for tapeworms, horses can be treated with either praziquantel or a cestocidal dose of pyrantel, and then 24 hour later fecals are performed (Sanada et al., 2009; Slocombe, 2006). If the horse was infected with tapeworms, there is a high probability that tapeworm eggs will be seen in the feces post treatment.

The research group led by Proudman at University of Liverpool developed the first fully validated and commercially available serological diagnostic test for diagnosing equine tapeworm infection (Proudman and Trees, 1996). This assay measures *A. perfoliata*-specific antibodies

and titer levels that have been found to correlate with worm burdens. However, being an antibody-based test, it more reflects exposure than actual infection, and horses can remain seropositive for months after treatment (Abbott et al., 2008). A different serological test is also available to test for the presence of antibodies to *A. perfoliata* at the University of Tennessee, but at present the test lacks sufficient validation as a quantitative assay for use in detecting current infections or for measuring worm burdens in individual horses. Most recently, a saliva-based ELISA has been validated for diagnosing tapeworms and made commercially available in the United Kingdom (Lightbody et al., 2016).

Because tapeworms are relatively common and widely distributed, have a strong seasonality of transmission, have potential to cause disease, and are difficult to diagnose, it is likely that a properly timed single annual tapeworm treatment would be beneficial for most horses. Even if this treatment is not needed for the health of an individual horse, a properly timed annual treatment given to all horses on a property should diminish transmission the following grazing season. However, there is no evidence that frequent tapeworm treatments throughout the year would provide any additional health benefit. Drug choices for treatment of tapeworms include praziquantel (licensed in the US for horses only in combination with ivermectin or moxidectin), or a cestocidal (double the nematode dose) of pyrantel pamoate. In most areas, this treatment should be given in the late fall or early winter after tapeworm transmission ends due to cold weather. It should also be noted that horses living in dry arid regions may have little or no exposure to tapeworms and thus would not require any cestocidal treatments. In these areas performing ELISA testing would be valuable, as low or negative titers would suggest that annual treatment is unnecessary.

### ***Parascaris* spp. (Roundworms; Ascarids)**

This parasite is the most important in foals causing ill-thrift and poor growth. Migrating larvae can cause signs of airway inflammation, including cough and nasal discharge (Clayton, 1986). Further, infection poses a risk for small intestinal impactions, which are associated with a guarded prognosis for survival, and can be further complicated by intestinal rupture (Cribb et al., 2006). Current evidence suggests that deworming of a heavily parasitized foal with an efficacious anthelmintic that has a paralytic mode of action can cause acute small intestinal impaction (Nielsen, 2016a). This association has not been found with benzimidazole type drugs, and these may therefore represent a better treatment choice for *Parascaris* spp. infections. The parasite is practically ubiquitous in breeding operations and the eggs are characterized by being particularly resistant to environmental influences and can remain present and infectious from year to year, if organic matter is present in the soil (Ihler, 1995).

*Parascaris* spp. infections may occasionally be diagnosed in immunocompetent adult horses, but clinical disease would be an extremely rare event. No specific evidence shows that the biology of *Parascaris* spp. has changed, but it appears empirically that more adult horses are positive for *Parascaris* spp. on fecal exams than in previous years. However, the increased fecal testing on farms may be rendering positive horses that previously went undiscovered.

High levels of resistance have been documented across the world to ivermectin and moxidectin (Peregrine et al., 2014), and some early findings suggest pyrantel and benzimidazole resistance

as well (Lyons et al., 2008b; Armstrong et al., 2014). Given that benzimidazoles have a non-paralytic mode of action, they appear to represent the best choice for *Parascaris* treatment on many properties, but pyrantel salts may be considered as well. Given the levels of resistance found to ivermectin and moxidectin on a large majority of farms, fenbendazole given at 10 mg/kg for five consecutive days may be the only remaining option for larvicidal treatment.

### ***Oxyuris equi* (Pinworms)**

Clinical disease from pinworms historically was seen mostly in young horses; however, in recent years cases in adult horses appear to be becoming increasingly more common (Reinemeyer and Nielsen, 2014). Pinworm infections tend to be sporadic, and usually only one or a few horses are affected out of a group. Clinical signs vary in intensity, but in severe cases, intense tail rubbing and hindquarter and/or perineal skin excoriations are seen. Some adult horses may have patent pinworm infections without showing any specific clinical signs. Definitive diagnosis is made by identifying the *O. equi* eggs. Eggs can sometimes be found on a fecal exam, but the scotch tape test or examination of perineal scrapings (using a tongue depressor and lube) are more sensitive. As a consequence of rubbing, horses can spread pinworm eggs throughout the horse's environment; transmission can occur in stalls and from contact with grooming materials, tail wraps, fence posts, etc. Furthermore, pinworm eggs are rather hardy, and can persist on the perianal region and in the environment for relatively long periods of time.

Apparent resistance to ivermectin in *O. equi* has been described in several recent reports and appears to be relatively common (Rock et al., 2013; Wolf et al., 2014; Sallé et al., 2016). If resistance is suspected, the available evidence suggests benzimidazoles should be given priority over pyrantel salts due to better historic efficacy levels (Reinemeyer and Nielsen, 2014). It should be emphasized that rectal lavage using liquid formulations of various anthelmintic products is very unlikely to have any effect as *O. equi* do not inhabit the rectum or descending colon.

Because the pruritus secondary to pinworm infections is caused by the material secreted by the female when depositing her eggs, washing the perineum and perianal region may help to relieve symptoms. After scrubbing, all materials should be discarded or washed in hot water with soap and/or disinfectants.

### **Bots (*Gasterophilus* spp.)**

Bots are rarely associated with measureable disease, but they can be aesthetically unpleasing. It is often recommended to treat with a boticide once each year during late fall or early winter as a clean-out treatment, which will help to decrease transmission in the next season. Currently, ivermectin and moxidectin are the only available parasiticides for horses with activity against bots. Recent data suggests variable efficacy of moxidectin against bot larvae (Reinemeyer et al., 2015).

## **METHODS OF PARASITE CONTROL**

### **Environment-based approaches**

Equine strongyle parasites begin life as an egg in a manure pile, which then must develop to infective larvae in the feces, get out onto the pasture, and then be ingested by a horse. Thus, infection of horses could be prevented if all feces were promptly removed from the pasture.

In a bygone era, the most elite stables employed pasture grooms, who followed grazing horses with a scoop shovel and a broom. Their job was to remove manure as quickly as it was dropped. In the 1980s, a similar approach was evaluated using updated technology. Studies at Newmarket in Great Britain examined the efficacy of cleaning horse pastures with a large commercial vacuum unit that was originally designed for golf course maintenance. Twice-weekly vacuuming was demonstrated to control pasture infectivity more effectively than routine deworming (Herd, 1986). However, the cost of the vacuum units was prohibitively expensive for the average horse owner, and the process only worked well on level, relatively dry pastures. Despite this, several commercial devices are now available for cleaning pastures, and these have found use on many horse farms.

### **Environmental Control**

Strongyle eggs hatch and develop into infective larvae under conditions of moderate temperature and moisture. Cold slows the rate of development or stops it altogether, and excessive heat kills eggs and larvae. It is possible to heat manure sufficiently to kill the parasites, including even ascarid eggs (Gould et al., 2012). Proper composting of manure and soiled bedding will generate relatively high internal temperatures, and strongyle larvae in manure are virtually eradicated by exposure to temperatures over 40 °C for a minimum of one week (Gould et al., 2012). Composting is a practice that should already be in place at any stable.

Non-composted horse manure should never be spread on pastures as this will increase the level of parasite contamination. This practice has been associated with higher *Parascaris* spp. prevalences in Germany (Fritzen et al., 2010).

Leaving pastures unoccupied for several months of the year may or may not reduce the risk of infection depending on the time of the year and the climate where the farm is located. Infective strongyle larvae (L3) can survive for only a few days to a few weeks in hot weather (temperatures around 40°C), but for as many as six to nine months during colder weather (Nielsen et al., 2007). Consequently, L3 survival in the environment will vary greatly from region to region and season to season. Thus, strategies for environmental control must be made based on local conditions.

Strongyle infective third-stage (L3) larvae can survive in wide extremes of weather and climate, but there are sets of conditions that are optimal and sets of conditions where development and/or survival are poor (Table 5). Therefore, it is recommended to focus anthelmintic treatments at times of the year that are most optimal for larval development, *i.e.* when transmission of strongyles is most likely. Doing so will reduce pasture contamination with infective stages, thereby decreasing the acquisition of new infections. In addition, a time when transmission is likely is also the time of year when adequate refugia are present, thus selection pressure for anthelmintic resistance is theoretically lessened. Conversely, it is recommended to avoid or limit treatments of equine strongyles during the winter months in cold temperate climates and during summer months in warm/hot climates (times of low refugia), in order to reduce the development of anthelmintic resistance.

**Table 5.** Effects of temperature on the survival, development and persistence of free-living stages (eggs, L1, L2, L3) of strongyles (Nielsen et al., 2007)

Development	Temperature Range	Survival
No development above this level	> 40 °C > 104 °F	Free-living stages die rapidly. Intact fecal balls may retain enough humidity to enable L3 to survive for some weeks.
Optimal temperature range for development of eggs and larvae. Reach infective L3 stage in as little as 4 days.	25 -33 °C 77 - 91 °F	Larvae survive on the shorter term (ie a few weeks), but conditions are too warm for long term survival
Eggs develop into L3 within 2-3 weeks.	10-25 °C 50-77 °F	L3 capable of surviving for several weeks to a few months
Lower limit for egg hatching is about 6 °C. At temperatures in this range, development will take several weeks to a few months.	6-10 °C 43-50 °F	L3 survive for many weeks and months under these circumstances
No hatching and no development	0-6 °C 32-43 °F	Eggs and L3 can survive for several months at temperatures just above the freezing point
No development during frost	< 0 °C < 32 °F	Developing larvae (L1 and L2) are killed, but unembryonated eggs and L3 can survive and persist for long periods ( <i>i.e.</i> months)
Alternation between freezing and thawing will usually not lead to development unless temperatures exceed 6 °C	< 0 > °C < 32 > °F	Repeated freeze-thaw cycles are detrimental to egg and larval survival

It is practically feasible to temporarily turn a grazing pasture into a hay field and recover the forage. Grazing infected pastures with ruminants may also assist in control (Eysker et al., 1986). Equine strongyle larvae are quite host-specific; they cannot infect cattle, sheep, goats or camelids. The only exception is the stomach worm, *Trichostrongylus axei*, which can infect both ruminants and equids, but this parasite has become very rare in equine establishments.

The environmental control of worms using nematophagous fungi has shown promising results. Several researchers have used these fungi that are harmless to people, and the environment (Larsen et al., 1999). Unfortunately, these biological control agents are not commercially available at present.

### **Alternative remedies**

An increasing number of so-called organic or herbal dewormers are appearing in tack shops and online, but the efficacy of these products has never been demonstrated in formal, controlled evaluations. These products exist primarily because they exploit differences in the labeling requirements for drugs vs. non-drug items. Before a drug can earn label claims for activity against parasites, this fact must be proven unequivocally to the Food and Drug Administration

by extensive efficacy and safety testing. Once a dewormer is approved by the FDA, the claims that can be made in advertising that drug are regulated by the FDA.

In contrast, products that are not considered drugs do not require FDA approval for marketing, so advertisers of non-FDA approved products can say just about anything they want, and their products do not have to be effective.

### **Anthelmintic Formulations Available**

**Benzimidazoles:** These drugs interfere with a worm's energy metabolism on a cellular level. They bind to beta tubulin and prevent its polymerization into microtubules. They are available in paste, liquid and pelleted formulations.

**Tetrahydropyrimidines:** Pyrantel pamoate and pyrantel tartrate act at the neuromuscular junction causing an irreversible rigid paralysis. Pyrantel salts only affect adult parasites that reside in the lumen of the gastrointestinal tract. Pyrantel pamoate is available in suspension and paste formulations, while pyrantel tartrate is formulated in alfalfa pellets and must be fed on a continual basis, serving only as a preventive, not a purge dewormer.

**Heterocyclic Compounds:** Several dewormers are classified as heterocyclic compounds, but piperazine is the only one used in horses. Piperazine works by depolarizing muscular membranes, which renders them resistant to the action of acetylcholine. The action of piperazine is limited to adult parasites. Piperazine is used infrequently in horses, and there is currently no formulation marketed for equine usage in the US, but one product is listed in Canada. It was available as a liquid or powder formulation which required nasogastric intubation.

**Macrocyclic Lactones:** These act on glutamate-gated chloride channels in nematode nerve and muscle cells, disturbing the normal transmission of nervous stimuli to muscles. The result is flaccid paralysis. Macrocyclic lactones are the most potent killers of worms, being effective at less than one-tenth the dosage of other classes of dewormers. They also have the unique quality of killing external parasites, such as lice, mites, and the cutaneous larvae of *Onchocerca*, *Habronema*, and *Draschia*. Macrocyclic lactones are available in paste (ivermectin) and as an oral gel (moxidectin).

**Isoquinoline-Pyrozines:** Praziquantel is the sole member of the isoquinolone class used in horses. It is also unique in that it has no activity against nematodes. Praziquantel is effective only against tapeworms. In North America, praziquantel is currently marketed only in combination with macrocyclic lactones, and the combination formulation is that of the parent compound (ivermectin if paste, moxidectin if a gel). Praziquantel is available as a single drug formulation in some European countries.

### **Parasite Control Programs: Points to Ponder**

Information about prepatent periods for the different parasites (*i.e.*, the period of time from ingestion of parasite infective stages until eggs are being shed in the feces), can be important for making decisions on when to treat. This information can be found in Appendix B.

### **Considerations for mature horses:**

Focus on control of cyathostomins. Depending on climatic conditions, one or two yearly treatments are sufficient to prevent occurrence of large strongyles. Consider including a treatment effective against encysted cyathostomins at a time when the mucosal burden is at its peak. Typically, this is more likely to occur towards the end of the grazing season, *i.e.*, fall in northern climates, and spring in the more tropical and subtropical climates. Include a cestocide at least annually if they are a problem in your region.

Deworming programs for adult horses should be designed with the following principles in mind:

- Evaluate the efficacy of the dewormers used on each farm at least every three years using the FECRT.
- A basic foundation of anthelmintic treatments should be considered to all horses. This should consist of one or two yearly treatments to target large strongyles, tapeworms, bots, and spirurid nematodes responsible for causing summer sores (*Habronema* spp. and *Draschia* spp.). In most cases, one or two yearly treatments will achieve this goal.
- All further treatments should be targeting horses with a high strongyle contamination potential.
- Focus anthelmintic treatments during seasons of peak transmission (usually spring and fall when pasture refugia are at their highest).

### **Considerations for foals, weanling, yearlings**

- Targeted treatments (selective therapy) based on FEC is NOT recommended in this age group. However, FECs are still important in this age group for other purposes and the following considerations should be made.
- During the first year of life foals should receive a minimum of four anthelmintic treatments. First deworming should be carried out at about 2-3 months of age, and a benzimidazole drug is recommended to ensure efficacy against ascarids. Second deworming is recommended just before weaning (approximately 4-6 months of age). An extra treatment can be justified before weaning if the time period between the two treatments exceeds 3 months. At weaning, FECs are recommended to determine whether worm burdens are primarily strongyles or ascarids, to facilitate the right choice of drug class. Third and fourth treatments should be considered at about 9 and 12 months of age, respectively, and treatment should primarily be targeting strongyles. In areas, where tapeworms are present, a tapeworm-directed treatment should be included in the 9-month treatment, or before the end of the first calendar year.
- Perform FECRT yearly to evaluate the efficacy of anthelmintics against strongyles and ascarids.
- *Strongyloides westeri* is rarely a cause of diarrhea in young foals thanks to the advent of the benzimidazoles and macrocyclic lactones. It is widely used to deworm mares around foaling to prevent the lactogenic transmission of this parasite. However, there is no evidence to support this procedure.



- Recently weaned foals should be turned out onto the “cleanest” pastures with the lowest parasite contamination levels.
- Yearlings and two-year olds should continue to be treated as “high” shedders, and receive about three-four yearly treatments with efficacious drugs, depending on the duration of the grazing season.

### **General points to consider**

- Do not under-dose horses and foals; use weight tapes or scales to determine body weights.
- Cyathostomins, large strongyles, and tapeworms are acquired on pasture. Ascarids and pinworms can be acquired in confinement as well as on pasture.
- Use properly performed FECs to determine shedding status and drug efficacy of new arrivals before turnout in common pastures.
- Consider using tapeworm serology (ELISA) submitted on at least 20% of resident herd members to determine exposure potential for tapeworms.
- Concentrate drug treatments when the local climate favors parasite transmission.
- Decrease strongyle-directed treatments when climate conditions are adverse (hot summer / freezing winter) for larval survival and / or transmission.
- Design a parasite control program that considers the farm’s management practices and climatic region. Consider the following:
  - Stocking density: Many horses and / or many different owners may make it more difficult and labor intensive to treat each horse as an individual. Heavy stocking rates resulting in a consistently high level of parasite exposure can challenge even the best deworming program.
  - Time horses spend on pasture: Limited access or the absence of grass often contributes to low FECs.
  - Age of horses on the farm: Are there foals/ weanlings/yearlings and / or mature adults. Treat youngsters as high shedders.
  - Is this an “open” herd: Institute a biosecurity program for all new arrivals that includes a FEC and larvicidal deworming prior to turn-out with resident horses.
  - What is the farm’s ability or willingness to “clean up” the environment using non-chemical means such as pasture rotation, cross-grazing with other species, manure removal and composting?

### **Summary for Parasite Control**

Given the information provided in this document, what is a rational worm control program? Worm control programs are best viewed as a yearly cycle starting at the time of year when worm transmission to horses changes from negligible to probable. Furthermore, it is critical that all treatment recommendations be viewed in the proper context. All treatment and non-treatment recommendations are made within the context of a preventive program where FEC surveillance is being performed. These recommendations, which are based on epidemiological principles, may not apply to individual horses on farms. Thus, if presented with a horse showing evidence of parasitic disease during the times of the year when treatments are not recommended (e.g., summer in south, winter in north), then this horse should be treated – and if

the horse is showing overt symptoms of strongyle-associated disease, a larvicidal treatment for targeting encysted cyathostomin larvae should be considered. Two recent blinded anthelmintic efficacy studies performed in the US with two different equine populations have documented a clear loss of larvicidal efficacy of the five-day double-dose fenbendazole regimen, whereas moxidectin had intact larvicidal efficacy in both studies (Reinemeyer et al., 2015; Bellow et al., 2018). Given the widespread occurrence of benzimidazole resistance in cyathostomin parasites across the world, moxidectin would be the treatment of choice in a large majority of locations.

It is important to keep in mind that these are just suggestions; thus, there are many variations of these suggested programs that would still meet the same goals and follow the same principles. Ultimately, each farm (with veterinary guidance) should develop its own program tailored to the specific needs of the farm and each animal. There is no such thing as a “one size fits all” program.

As outlined, all adult horses should benefit from a basic foundation of one or two treatments per year. Low strongyle egg shedding horses with naturally strong immunity to cyathostomins will need no other treatments because the two treatments have covered the needs of the other parasites and these horses are generally protected naturally from disease caused by cyathostomins by their immune state. In traditional deworming programs, repeated treatment of low shedder horses every 2-3 months accomplishes little to improve their health, but it does promote drug resistance. Moderate and high egg shedders will need a third and sometimes a fourth treatment for cyathostomins.

Any additional treatments would be given on an “as needed” basis depending on whether a specific parasitic infection or disease is diagnosed. For example, if *Anoplocephala* eggs are seen when performing FEC, a second tapeworm treatment during the year might be warranted. Likewise, if pinworms are diagnosed, any horse showing symptoms should be treated with an effective anthelmintic. Ivermectin and moxidectin remain the foundation for control of strongyle parasites, although signs of emerging resistance have been reported. In contrast, resistance to these drugs is common in *Parascaris* spp. and *Oxyuris equi*.

Strongyle resistance is well documented against pyrantel, fenbendazole, and oxibendazole, and these drugs can therefore only be used if a FECRT has documented good efficacy. In addition, resistance in *Parascaris* spp. is still uncommon for these drugs, thus these are often solid choices when targeting this parasite.

**Developed by the AAEP Parasite Control Subcommittee of the AAEP Infectious Disease Committee in 2013.** Original subcommittee members included: *Martin K. Nielsen, DVM, Ph.D., Dipl. EVPC (chair), Linda Mittel, MSPH, DVM, Amy Grice, VMD, Michael Erskine, DVM, Dipl. ABVP, Emily Graves, VMD, Dipl. ACVIM, Wendy Vaala, VMD, Dipl. ACVIM, Richard C. Tully, DVM, Dennis D. French, DVM, Ph.D, Dip. ABVP, Richard Bowman, DVM, Ray M. Kaplan, DVM, Ph.D, Dipl. ACVM, Dipl. EVPC.*

**Reviewed and updated in 2019 by the AAEP Infectious Disease Committee with additional review by the original subcommittee above.**

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## **Appendix A: Egg counting techniques**

### **Modified McMaster Fecal Egg Count (FEC) Procedure**

The method described below has a detection limit of 25 EPG, which makes it useful for identifying high egg shedders, but less appropriate for the FECRT.

#### **Supplies needed:**

- Disposable paper cup (Dixie cup) or small container for feces
- Small strainer (household)
- Pipette, eye dropper or syringe to dispense fecal solution
- Cheese cloth or gauze sponge
- McMaster Slide
- Flotation medium

#### **Procedure Steps:**

1. Weigh out 4 g of feces in a small container or paper cup.
2. Add 26 mls of flotation medium (to bring the volume up to 30 ml) to feces. Mix well.
  - a. Note: If you do not have a scale, you can add feces to the 26 ml of solution and when the volume reaches 30 mls, you have added 4 g.
3. Strain through one or two layers of cheesecloth, one layered gauze squares, or tea strainer), mix well.
4. Mix the sample well and then immediately withdraw about 1 ml of the suspension with a pipette or syringe and fill the first counting chamber of the McMaster slide.
  - a. Repeat the process to fill the second chamber.
  - b. Let the slide stand for two to five minutes to allow eggs to float to top.
    - i. If visible air bubbles are present, the chamber should be emptied and refilled.
5. Steps three and four should be done at the same time without letting the sample sit between steps, since eggs are in flotation fluid and will immediately begin to rise to the top of the fluid. You want to be sure to get a representative sample of the mixed solution.
6. Once chambers are filled, step three can be started for the next sample.
7. Once filled, the chambers can set for 60 minutes before counting without causing problems if using sodium nitrate. Longer than this and drying/crystal formation can begin. With sodium chloride, crystal formation occurs much more quickly.
8. Count all eggs inside of grid areas (only count the eggs which have more than half of their area inside the outer lines of the grid) at 100x total magnification (10x ocular lens and 10x objective lens). Focus on the top layer, which contains the very small air bubbles (small black circles). Count both chambers.
  - a. Count only strongyle eggs (oval-shaped, about 90 microns long). Ascarid eggs (round, about 80-90microns long) can also be counted, but should be counted separately from the strongyle eggs. Do not count *Strongyloides* (oval, about 50 microns long), tapeworm eggs (D-shaped), or *Eimeria leuckarti* (large brown oocysts of the same size as strongyle eggs)--only notations are made as to the presence of these other parasites.
9. Multiply the number of counted eggs in each category by 25 to achieve the number of eggs per gram (EPG) per egg type

## **Modified Wisconsin Technique**

This technique does not require specialized slides, but involves centrifugation using a swing-bucket rotor. The detection limit is 1 EPG, which makes the technique very suitable for the FECRT.

### **Supplies Needed:**

- Small strainer (household)
- Pipette, eye dropper or syringe to dispense fecal solution
- Cheese cloth or gauze sponges
- Test tube - 15 mls or centrifuge tube - 15 mls
- Centrifuge – swinging head centrifuge not fixed
- Sheather's solution (sugar solution)

### **Procedure Steps:**

1. Weigh out 1 g fecal sample in a small beaker (50-100 ml).
2. Add 20 ml of tap water in the fecal material.
3. Stir very well with a spatula and mash the material until it is completely broken apart.
4. Pour the mixture through the funnel with one layer of cheesecloth (or tea strainer) into another beaker (150-250 ml), stirring the material in the funnel while pouring. Press the material remaining in the funnel with the spatula until nearly dry.
5. Add 10 ml of tap water to the beaker and rinse into a mixture the material clinging to the sides and bottom, and then pour this mixture through the material in the funnel, stirring the material in the funnel while pouring. Press the material in the funnel until dry again, and then discard.
6. Stir the material in the beaker and immediately pour the contents of the beaker into two 15 ml tubes, being careful to divide it as equally as possible. There should not be any material left in the beaker.
7. Centrifuge the tubes for 5 to 7 minutes at 300 g to pull fecal debris to the bottom of tube.
8. Pour off the supernatant, leaving the pellet at the bottom of the tubes.
9. Fill the tubes to just over the top with Sheather's solution and place a cover slip onto the meniscus.
10. Centrifuge at 300 g for 10 minutes.
  - a. Note that if a swing-bucket rotor is not available then a fixed-angle rotor can be used, but cover slips may fall off. If using a fixed-angle rotor the procedure should be modified as follows: the tube is initially filled only  $\frac{3}{4}$  fill with Sheather's solution and then after centrifugation the tube is filled with Sheather's solution until a positive meniscus forms. Then a coverslip is placed on the tube and the tube is left to sit for 10-15 minutes before removing the coverslip and placing it on a slide for counting.
11. Let sit for about 5 minutes, and then remove the cover slip and place on a slide.
12. Examine the entire cover slip from both tubes and count the number of eggs that you find.
13. The number of eggs counted equals the EPG as the detection limit is 1 EPG.



## Appendix B: Prepatent periods of important equine parasites

Species	Prepatent period	References
Cyathostomins	2-3 months	(Round, 1969)
<i>Parascaris equorum</i>	2½-3 months	(Clayton and Duncan, 1977)
<i>Anoplocephala perfoliata</i>	1½-4 months	(Bain and Kelly, 1977)
<i>Strongylus edentatus</i>	11-12 months	(Enigk, 1970)
<i>Strongylus vulgaris</i>	6-7 months	(Enigk, 1970)

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