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Investigations into the Incidence and Control of Selected Parasites and Pathogens which Infect Arkansas Horses

Stephanie Ann O'Berg Rainbolt
University of Arkansas, Fayetteville

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Stephanie Rainbolt
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_____
Dr. Tom Yazwinski
Thesis Director

_____
Dr. Nancy Jack
Committee Member

_____
Dr. Jeremy Powell
Committee Member

ABSTRACT

Parasite control is an important aspect of health management of horses, particularly the control of gastrointestinal (GI) parasites. Recently, treatment recommendations have involved selective use of anthelmintics in horses with fecal egg counts (FEC) greater than a specified threshold. The objectives of this study were; (1) to determine the prevalence of helminths in our area by egg and L3 determinations, (2) to determine if certain horses maintained low FEC, therefore eliminating the need to treat them on a year-round basis and (3) to determine the effectiveness of four common treatments (moxidectin, ivermectin (pioneer and generic), fenbendazole and pyrantel tartrate) via a standardized fecal egg count reduction (FECR) test. Fecal samples were collected from 226 horses at 14 different farms with a total of 933 fecal samples and 259 coprocultures evaluated over eight months. A treatment threshold of FEC > 200 eggs per gram (EPG) was used and horses were sampled once, twice, or three times over the course of the study. Of the 933 fecals analyzed, 303 had an EPG of zero, 407 were < 200 EPG, and 223 were >200 EPG. There were 37 samples that contained eggs other than Strongyle-type eggs and at least one of the three major large strongyle species were found in seven of 259 coprocultures evaluated. During the study, 125 horses were treated and 101 horses did not require treatment. Of these, six of the horses were sampled three times without exceeding the treatment threshold. The efficacy of both moxidectin and ivermectin was high (98.5% and 99.7% FECR), fenbendazole was expectedly low (57.7% FECR), and the efficacy of pyrantel was difficult to elucidate since all treatments were preceded by moxidectin. These results are consistent with other published studies that suggest using selective treatment to control equine GI parasites.

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DEDICATION

This thesis is dedicated to Evelyn Cloninger and Rusty Rainbolt, none of this would be possible without their support.

TABLE OF CONTENTS

I. Literature Review

Parasite control, particularly of gastrointestinal parasites, is an important aspect of the overall health management of horses. An understanding of the prevalence and pathogenicity of the parasites offers insight towards effective management programs utilizing both drugs and husbandry techniques. Six groupings of internal parasites that are of main concern, due to either pathogenicity or prevalence, include; small strongyles, large strongyles, tapeworms, bots, pinworms, and ascarids.

a. IMPORTANT EQUINE PARASITES

Small Strongyles

Cyathostomes (small strongyles) are the most prevalent intestinal parasites in horses around the world. Currently, there have been 83 different species of nematodes identified that infect horses. Of these, 50 are cyathostomes and are commonly referred to as small strongyles. A typical small strongyle infection includes thousands of adult and larval stage nematodes, and is comprised of 5 to 10 of the most prevalent species (Lichtenfels et al., 2008).

Cyathostomes have a typical “trichostrongyle” life cycle, with adult females depositing eggs in the cecum and large intestine, which are passed with the feces into the environment. Favorable environmental conditions allow eggs to hatch within one week, although this can take up to four weeks (Reinemeyer, 1986). Once the first stage larvae (L1) have emerged from the egg, they live on dissolved nutrients, undergo a molt and develop to second stage larvae (L2). These larvae in live on a nutrient-rich feces and molt to the third stage, infective larvae (L3). The L3 migrate from the feces and can survive on pasture for up to 11 weeks in the winter, but only 2-3 weeks in the dry summer months (Reinemeyer, 1986). Once L3 have been ingested by the equine host, prepatency usually lasts for 5-6 weeks, although a prepatent period of up 8 weeks has been observed (Klei and French, 1998). Inhibition of the parasitic L3, which occurs in the cysts in the mucosa or submucosa of the large intestine, can be influenced by season, infection levels, and acquired immunity by the host. These cysts can endure for up to 3 years in older horses (Klei and French, 1998). During non-inhibited development, the L3 develop to fourth stage

larvae (L4) within 6-12 days in the cysts and move into the lumen, where they further develop into adults, with 50-55 percent residing in the large colon. A large portion of the pathogenicity of cyathostomes is due to larval cyathostomiasis, a condition in which a large number of L4 emerge from cysts in the large intestine and cecum, causing severe colitis, diarrhea, and possibly death, especially in younger animals (Klei and French, 1998).

Large Strongyles

Large strongyles are the most pathogenic nematodes that infect horses, and are arguably the most pathogenic of all parasites in horses. There are three species of the genus Strongylus that are the most important large strongyles in horses. These species are *Strongylus vulgaris, S. equinus,* and *S. edentatus* (Lichtenfels et al., 2008). The large strongyle life cycle is dissimilar from that of small strongyles, with a prepatent period that is normally six months to one year, depending on the species present. Adult large strongyles are found in the cecum and colon; however, they are attached to the wall of the intestine and suck blood, damaging mucosa in the process; thereby giving these parasites the common name of blood worms. The other prominent difference between small and large strongyles is the migration of the larval stages of each of the large strongyles. Each of the three species of large strongyles has a unique migratory path going from the gut, to various organs and then back to the gut. Small strongyle larvae simply migrate in the mucosa of the cecum and large intestine.

The pathogenicity of large strongyles is primarily due to the migration of the larvae, usually the L4 stage, before development into adult worms. *S. vulgaris* is the most pathogenic species of large strongyles, with migration occurring primarily into the cranial mesenteric artery. After ingestion of the L3 larvae by the equine host, development of L4 larvae occurs, followed by migration through the wall of the small intestine, cecum, or ventral colon, into the arterioles, then into small arteries, upstream to the larger arteries, and eventually to the cranial mesenteric artery. Larvae are passed back to the cecum or large colon where they form nodules in the walls of the intestine. Adults are only sexually mature upon leaving these nodules; a process that takes approximately 6 months after ingestion of L3 larvae (Drudge, 1978). *S. edentatus* does not possess the high pathogenicity of *S. vulgaris* because the migration of L4 larvae

occurs primarily in the liver rather than arteries. Ingested larvae move through the cecum, through portal veins to the liver, through the peritoneal lining of the abdominal cavity, and then back through the intestinal wall to the mucosa. This migration period, from ingestion to development of adults moving into the mucosa of the ventral colon, requires approximately 11 months (Drudge, 1978). The migration of *S. equinus* is very similar to that of *S.edentatus*; however, once the larvae leave the liver they also travel to the pancreas before returning to the mucosa of the cecum. The development of this less common large strongyle takes approximately nine months from ingestion of larvae to development of adults in the cecum (Drudge, 1978).

Tapeworms

Cestodes (tapeworms) are increasingly thought to be an important gastrointestinal parasite in horses, with research into the correlation of infections with colic, or intestinal disturbances (Proudman, 2003). There are three species of tapeworms with importance in horses, *Anoplocephala perfoliata, A. magna,* and *Paranoplocephala mamillana*. Each species resides in a distinct location of the intestinal tract, and pathogenicity of these parasites is dependent on both their location and infection rate in horses (Lyons et al., 2006). The most common species, *A. perfoliata*, is thought to be the most pathogenic because it resides near the ileocecal junction, leading to incidences of spasmodic colic and cecal ulcerations, with the potential of death of animals with heavy infections. *A. magna* is the largest of the three species, but is relatively uncommon and resides in the posterior small intestine. The smallest species is *P. mamillana*, which is found in the anterior small intestine or stomach, and is also relatively uncommon (Lyons et al., 2006).

The life cycle of tapeworms is indirect, with orbatid mites serving as intermediate hosts for the infective stages. The entire life cycle requires approximately four to six months, with a two to four month period of development in the intermediate host and two months for development in the definitive host (Drudge, 1978). The mite ingests embryonated eggs from the environment and the cysticercoid (larval stage) develops in its body cavity. Horses ingest infected mites on pasture, and the larvae develop into adults in the intestinal tract. The scolex of the adult attaches to the horse's intestinal wall and maturation

occurs through the growth of the strobila from the “neck” of the tapeworm towards the posterior intestine of the host. The proglottids that make up the strobila each contain male and female reproductive systems resulting in proglottids full of eggs (gravid). These gravid proglottids pass with the feces into the environment, releasing eggs for mites to ingest (Lyons et al.,2006).

Bots

“Bot” is the common name for the maggot stage of the bot fly that infects horses. There are several species of the genus *Gastrophilis* that infect horses; with each “colonizing” a different location on the stomach mucosa. The two most common species are *G. nasalis* and *G. intestinalis*. Pathogenicity is due to the pits formed in stomach tissue, as well as occasional perforation and peritonitis. Adult flies in the environment mate and the females cement eggs (“nits”) containing first stage larvae on the hairs covering the horse’s body, concentrating on the legs, shoulders, and neck. Dependent on the species, eggs either hatch spontaneously after one week or are stimulated to hatch by the horse licking or chewing on the area containing the eggs. First-instar larvae migrate through oral tissue and develop into second-instar larvae in three weeks. The second-instar larvae migrate to the back of the throat and are swallowed, passing to the stomach where development into third-instar larvae occurs after three to four weeks. The third-instar larvae create pits in the lining of the stomach, where they can remain for up to 10 months before detaching and passing into the environment with the feces. Upon entering the environment, the larvae burrow into the ground to pupate for approximately one to two months. Adult flies emerge, mate, and females lay eggs for approximately two months prior to their demise (Drudge, 1978).

Pinworms

Pinworm infections are found in all ages of horses, and are important because of the irritating effect they have on the host (“indirect”) pathogenicity. The common pinworm is the species *Oxyuris equi,* which is found in the large intestine. Females migrate to the anus, where they rupture and deposit eggs around the perianal region of the horse. The development of infective larvae in the eggs requires three to five days. Upon ingestion by the host, larvae develop into fourth stage larvae within three to 10 days.

Fourth stage larvae develop into sexually mature worms over five months as they are attached to the mucosa of the large intestine. Irritation to the host is due to the migration of the females out of the anus and their subsequent rupture. Egg deposits dry on the horse's skin, which causes severe pruritis around the tail head and can cause secondary bacterial infections from horses rubbing their tail against any available surface. Horses can sustain an infection of over 20,000 pinworms with no obvious, specific clinical signs other than tail rubbing (Drudge, 1978).

Ascarids

Parascaris equorum (ascarids) commonly infect young horses, particularly those under one year of age. The pathogenicity of ascarids is due to the possible rupture of the small intestine, and possible damage in the liver and lungs from large numbers of migrating larvae. Adult ascarids reside in the small intestine and are the largest nematode parasites of horses. Individual females can lay up to 200,000 eggs per day, which pass with the feces into the environment and become infective in two weeks. Infective eggs remain in the environment for many years in a resistant shell, and hatch upon ingestion by the equine host. Larvae released from the eggs migrate through the intestinal wall, through portal veins to the liver, and into the lungs. Immature larvae are coughed up and swallowed, move to the small intestine, and develop into mature adults. The entire life cycle requires four months, with migration and development in the host requiring three months (Drudge, 1978; Lyons et al., 2006).

b. CONTROL OF IMPORTANT PARASITES

Chemical control of parasites is an important part of the overall health management program for horses. Anthelmintic use should be primarily based upon the helminth incidence and the drug's spectrum of activity. There are currently three classes of anthelmintic compounds in use for the treatment of equine gastrointestinal nematodes; macrocyclic lactones, tetrahydropyrimidines, and benzimidazoles. Praziquantel (quinoline class) is also used for the control of tapeworms; however, it is only marketed in combination with macrocyclic lactones.

Macrocyclic Lactones

The macrocyclic lactone class of anthelmintics includes two subclasses of compounds, milbemycins (including moxidectin) and avermectins (including ivermectin), both of which cause flaccid paralysis of the nematode by interfering with neurotransmission and muscle cell function (Wescott, 1986). Moxidectin and ivermectin are nearly identical in chemical structure, but moxidectin lacks a sugar group that is contained on the ivermectin compound. This alteration gives moxidectin exceptional lipophilic properties, enabling it to target encysted cyathostomes (late L3/L4 mucosal cyathostome larvae). Both moxidectin and ivermectin are labeled for the control of bots, adult large-mouth stomach worms, pinworms, ascarids, adult and L4 small strongyles, large strongyles, and adult hairworms (*Trichostrongylus axei)* (Brady and Nichols, 2009).

Tetrahydropyrimidines

Tetrahydropyrimidines (pyrantel salts) include pyrantel tartrate and pyrantel pamoate. Both of these compounds are approved for the control of mature infections of large strongyles, small strongyles, pinworms, and ascarids. The pyrantel salts cause nematode paralysis by stimulated release and maintenance of acetylcholine at neuron synapses (Brady and Nichols, 2009). Pyrantel tartrate usage is recommended after horse treatment with a larvacide, such as moxidectin, and is administered at a low daily dosage. Daily pyrantel has also been shown to control tapeworm infections (Kivipelto et al., 1998). Pyrantel pamoate at a triple dose has also been shown effective against tapeworms (Kivipelto et al., 1998) and has been approved and labeled for double dosage use for the control of cestodes (Phoenix, 2005).

Benzimidazoles

Benzimidazoles have been on the market longer than the other two classes of anthelmintics, spanning over fifty years of use by way of multiple formulations. Currently in the horse industry, the two compounds used most often are oxibendazole and fenbendazole. Fenbendazole is labeled against ascarids, pinworms, small strongyles, and large strongyles, as well as encysted small strongyle larvae when given at a double dose for five consecutive days (Brady and Nichols, 2009). Benzimidazoles act on

nematodes through interference of metabolism by microtubule inhibition (Roberson, 1977; Rew and Fetterer, 1986).

c. RESISTANCE TO EQUINE ANTHELMINTICS

Anthelmintic resistance is a cause for concern and is the result of frequent use of anthelmintics in the horse industry. The most common ways to measure efficacy of deworming products are the use of fecal egg counts (FEC), egg reappearance periods (ERP), and fecal egg count reductions (FECR). There is a lack of consistency with the measurement of resistance, leading to conflicting reports of its prevalence in the equine industry. The World Association for the Advancement of Veterinary Parasitology (WAAVP) defines resistance as a FECR percentage that is less than 95% (Coles et al., 1992). Analysis methods of FECR tests differ among researchers and the accuracy of some methods has been questioned, although no consensus has been achieved (Denwood et al., 2010).

The development of resistance to all of the major classes of anthelmintics has been associated with several factors. The primary factor contributing to resistance has been the high frequency of treatment, particularly with only one compound or class of anthelmintic (van Wyk, 2001; Brady and Nichols, 2009). Underdosing is also a factor in resistance development (Brady et al., 2008), along with a low presence of refugia maintained on farms. Refugia is defined as the population of nematodes that remain unexposed to chemical compounds, i.e., free-living populations on pasture, animals not treated with the compound, or encysted larvae not exposed to the anthelmintic (van Wyk, 2001; Brady and Nichols, 2009).

Benzimidazoles are the most common anthelmintics with documented resistance, particularly with cyathostome populations. Although resistance has been documented over several decades (Little et al., 2003) and in multiple countries (Kaplan, 2002), fenbendazole remains one of the most popular anthelmintics in use today (Brady and Nichols, 2009). Multiple studies have shown the efficacy of fenbendazole to be extremely low, with FECR percentages of 84.4% (Varady et al., 2004), 65.1% (Varady et al., 2000), -36% (Rossano et al., 2010), and no significant reduction in FEC (Martin-Downum et al.,

2001; Chandler and Love, 2002). According to Kaplan (2002), benzimidazole-resistant cyathostome populations have greatly overwhelmed the populations of susceptible cyathostomes, leaving the majority of farms with only resistant strains.

Tetrahydropyrimidines have been shown to be resisted by cyathostomes in many countries. The prevalence of resistance to this compound has not been as widespread as resistance to benzimidazoles, perhaps due to the fact that it has not been on the market as long (Brady and Nichols, 2009). Pyrantel tartrate, given as a daily top-dressing, may be responsible for the development of resistance in this class (Kaplan, 2002). No published data reveals this direct correlation; however farms with documented pyrimidine resistance have also had a history of daily pyrantel tartrate use (Tarigo-Martinie et al., 2001; Kaplan, 2002). Research conducted on pyrimidine resistance is generally correlated with resistance to benzimidazoles, which could indicate cross-resistance (Lyons et al., 2001; Brady and Nichols, 2009). Although resistance has been documented with cyathostomes, pyrantel pamoate has been shown to remain effective against *Oxyuris equi* infections (Reinemeyer et al., 2010a).

Macrocyclic lactone resistance has been documented for ascarids, but has not yet been shown in cyathostomes. In a 2002 review article, Kaplan reported no findings of ivermectin resistance; however researchers did report ascarid resistance to ivermectin (Boersema et al., 2002; Brady and Nichols, 2009; Reinemeyer, 2010b). In the United States, there is currently no evidence of cyathostome resistance to milbemycins, and recent studies show efficacies of moxidectin to be 99.9-100% (Chandler and Love, 2002), 99.1% (Martin-Downum et al., 2001), and 100% (Rossano et al., 2010); however, the ERP for moxidectin was shorter than previously reported according to Rossano and colleagues (2010). Other research has demonstrated ivermectin to be effective against cyathostomes, with a reported efficacy of >99% (Klei et al., 2001).Efficacy against *Oxyuris equi* has also been shown at 96% for adults and >99% for fourth stage larvae (Reinemeyer et al., 2010a).

d. PARASITE MANAGEMENT

Deworming Protocols

Research has been conducted in various EU countries to document how horse owners and veterinarians are controlling parasites. In the UK in the late 90's, horse owners said they used rotational deworming practices and were influenced in their decision to do so by advertisements and magazine articles and occasionally their veterinary surgeon (Lloyd et al., 2000). This process was confirmed by Allison et al (2011), who found that 50% of horse owners receive their deworming advice from a veterinary surgeon, and about 30% used professional advice to develop a selective deworming protocol. In Ireland, only 54% of horse owners devised their deworming protocols based on veterinary advice, and none of them used selective deworming (O'Meara and Mulcahy, 2002). In Denmark, where anthelmintics have been available only by prescription since 1999, veterinarians are responsible for determining when a horse needs treatment. Most veterinarians (97%) reported using fecal egg counts to guide their treatment decisions, but in cases of foals or horses with "suspicion of clinical parasitic disease" fecals were not performed prior to treatment (Nielsen et al., 2006). This same group of veterinarians also reported low utilization (11% of practitioners) of fecal egg count reduction tests to determine anthelmintic efficacy and resistance (Nielsen et al., 2006).

The use of proper deworming protocols is an important aspect of internal parasite control. In the past, a practice known as 'interval deworming' was recommended by parasitologists (Drudge and Lyons, 1986). This practice called for the treatment of horses every 6-8 weeks, primarily targeting the removal of *Strongylus vulgaris* in order to prevent verminous colic (Drudge and Lyons, 1986; Kaplan, 2002). This strategy has been widely implemented, and strictly followed, since its introduction (Kaplan, 2002). Interval dosing has been extremely successful in controlling *Strongylus spp.* but it has led to resistance by cyathostomes, which are now considered the most important internal parasite in horses (Duncan and Love, 1991; Larsen et al., 2011).

In order to address the resistance of cyathostomes, parasitologists have begun to implement new treatment strategies. Selective treatment and rotational deworming have been examined in many studies around the world, and both have been shown to be effective on horse farms that harbor resistant

parasites (Duncan and Love, 1991; Gomez and Gorgi, 1991; Brady et al., 2008; Becher et al., 2010; Larsen et al., 2011).

Rotational deworming has been studied as a strategy to regain effectiveness where resistance by certain parasites has been documented (Brady et al., 2008). Following a fast rotation between different classes of anthelmintics, fenbendazole (10 mg/kg for five days) was shown to have an efficacy of 98.7% in mature horses on a farm with documented benzimidazole resistance (Brady et al., 2008). Reinemeyer et al (2010b) found that foals infected with ML-resistant strains of *P. equorum* could be treated with pyrantel pamoate and have a significant reduction in adult worms. Although researchers have suggested it, additional research on fast vs. slow rotation has not been published (Kaplan, 2002; Brady et al., 2008). Recommendations of rotation between drug classes are numerous, with an agreement that only effective anthelmintics be used (Nielson et al., 2010, Reinemeyer et al., 2010b). Additionally, a recommendation of rotation based on parasite prevalence by season has been forwarded (Nielson et al., 2010), but there have not been studies published to confirm or refute these suggestions.

The most novel approach to equine deworming is selective treatment, which is a program based upon diagnosing internal parasites in horses, and then treating individual horses based upon that diagnosis (Kaplan, 2010). Usually, this diagnosis is made by performing fecal egg counts on all horses and then treating only those over a certain threshold (Gomez and Georgi, 1991). The selective treatment protocol has been used in small ruminant production with some success, with treatment criteria based on the use of FAMACHA or production characteristics such as weight gain, milk yield, or wool yield (Kenyon et al., 2009; Gaba et al., 2010). In horses, the use of selective treatment has been implemented in the European Union through regulation of deworming products, which are only available with a prescription from a veterinarian (Anderson et al., 2012).

While various studies have confirmed that selective treatment helps maintain efficacy of current drugs (Duncan and Love, 1991; Gomez and Georgi, 1991; Becher et al., 2010; Larson et al., 2011), there have not been any definitive studies on when fecal samples should be taken, or any that prove that selective deworming aids in actually reducing resistance. In 2010, Becher and colleagues found that out

of 129 horses sampled each month for 10 months, only 29.5% needed treatment (FEC >250 EPG). This study demonstrated that a significant decrease in the number of treatments can be obtained, thereby maintaining refugia and potentially decreasing the selection pressure for development of resistant parasites (Becher et al., 2010). In the United States, there have not been recent studies to confirm the selective treatment data coming from the EU; however, this could be due to the fact that the American Association of Equine Practitioners (AAEP) has only recently recognized selective treatment as a protocol. The new (2013) AAEP recommendations for deworming programs include different guidelines for horses under 3 years of age versus horses over 3 years of age, with more traditional guidelines for treating young horses (every 3 months) to control *P. equorum* and prevent disease associated with large strongyles. The guidelines for older horses recommend the use of fecal egg counts and fecal egg count reduction tests to ensure that only indicated horses receive treatments and the drugs in use maintain their effectiveness (Nielsen et al., 2013).

II. Introduction

The presence of gastrointestinal parasites can reduce animal health and body condition. In horses, this is indicated by a poor hair coat, diarrhea, poor body condition and in some cases, colitis (Drudge and Lyons, 1986). As clinical signs are not definitive for parasitism, fecal flotations are performed to confirm parasite burdens in poorly performing animals. Quantitative flotations give fecal egg counts (FEC), measured in eggs per gram (EPG), and constitute the most effective tool for parasitological interpretations in live animals. The flotations show the eggs shed in the feces by mature helminths residing in the digestive tract, which are in turn used as an indication of the population in the horse. Fecal egg counts are generally performed only when there is already suspicion of infection and treatment has already been recommended. Commonly, treatment is given preemptively to healthy animals in order to prevent the development of clinical signs (Kaplan, 2002).

Anthelmintic drugs are used to control parasite infections and several are currently on the market for use in horses. Historically, fenbendazole has been one of the most commonly used anthelmintics in the United States, but ivermectin is probably the most popular anthelmintic today (Chandler and Love,

2002). The newest drug on the market, moxidectin, is also commonly used although it is contraindicated for use in foals younger than 6 months of age or severely debilitated horses due to its lipophilic properties. Moxidectin can be used therapeutically in conjunction with pyrantel tartrate, which is given at a daily larvacidal dose in the feed. The drugs used for anthelmintic treatment should be dependent upon the efficacy and farm-specific protocol.

Several protocols of anthelmintic intervention have been utilized by equine caretakers, with interval treatment the most common. Interval treatment calls for the use of anthelmintics every 6-8 weeks in horses sharing a pasture, regardless of parasite burden. This protocol has led to the development of resistance, particularly by small strongyles (Larsen et al., 2011). Exposure of entire populations of helminths to particular chemicals results in establishing a parasitic gene pool of only those resistant to the drug. In requiring the treatment of all animals, interval dosing exposes all parasites to the drugs used on that particular farm. Resistance to the most commonly used drugs in the equine industry has been thoroughly documented in multiple countries and is often correlated with interval dosing protocols (Kaplan, 2002). In order to prevent the extreme resistance currently found in small ruminants, equine veterinarians have begun to recommend different protocols (Kenyon et. al, 2009; Nielsen et al., 2013).

Selective treatment has been gaining ground in veterinary parasitology; however, its use in the field has not been thoroughly documented or evaluated. Various methods have been implemented in selective treatment, with the use of fecal flotations to distinguish two groups of horses on each farm as the basis of this protocol. One group of animals, the high-shedding horses, is treated with an anthelmintic while the others, the low-shedding horses, are left untreated. Determination of treatment is based on a pre-selected threshold, generally between 200-250 EPG. The untreated animals help to maintain refugia (a population of the parasites not exposed to the drugs); a biological means of diluting the gene pool of those helminths resistant to chemicals (van Wyk et al., 2001). This can help reduce the rate/degree of resistance, which in turn can improve anthelmintic efficacy.

Efficacy of commonly used drugs has been severely depressed by resistance in small strongyles. Small strongyles are the most common gastrointestinal parasites found in horses and are responsible for

the majority of eggs found in a fecal egg count; and proportionally greatly determine the treatment threshold (Love et. al, 1999). Large strongyles are also found in the FEC but their eggs are similar in size and shape to the small strongyles and are therefore not differentiated in flotations. However, identification can be made through the use of coprocultures, larval harvest, and larvae identifications (Ivens, 1978). The FEC is used to estimate efficacy of anthelmintics by performing flotations at the time of treatment and again 14-21 days post-treatment, comparing the egg counts. This is known as a fecal egg count reduction test (FECRT) and is presented as a percentage of efficacy. Drugs are considered to be efficacious with ≥95% FECR. Selective treatment could help maintain efficacy by reducing resistant populations of parasites through monitoring parasite burdens with FEC and the FECRT (Larsen et al., 2011).

The objectives of this study were; (1) to determine the prevalence of helminths in our area by egg and L3 determinations, (2) to determine if certain horses maintained low FEC, therefore eliminating the need to treat them on a year-round basis and (3) to determine the effectiveness of four common treatments (moxidectin, ivermectin (pioneer and generic), fenbendazole and pyrantel tartrate) via a standardized fecal egg count reduction test.

III. Materials and Methods

Timeline

This study was conducted from February 2011 through October 2011.

Horses

Fecal samples were collected from 226 horses housed on 14 farms in Northwest Arkansas, Central Arkansas, and the University of Missouri in Columbia. Selected farms had to maintain a herd of at least 10 horses for the duration of the trial. At the beginning of the study, horses ranged in age from 8 months to 35 years and included 99 mares, 126 geldings, and one stallion. There were 39 breeds represented at the farms. On-going farm management procedures, with the exception of anthelmintic treatments, were kept in force at each farm for the study.

Fecal Samples

Fecal samples were collected from each animal at pre-treatment (PRT) (day -7 to day 0) and at post-treatment (PT) (3-5 weeks following treatment). Re-treatment and re-sampling was separated by approximately 3 months. Eighty-nine horses were sampled/treated once, 116 horses were sampled/treated twice, and 21 horses were sampled/treated three times. Samples from the horses were taken either rectally or collected from individual paddocks or stalls and refrigerated at 5°C until examination within 2-5 days following collection. Fecal samples were quantitatively examined using single centrifugation of 1 g feces in saturated $MgSO_4$ (Martin-Downum et al, 2001). Coprocultures were also conducted for samples with a FEC ≥ 20 EPG for the first 6 months and ≥ 50 EPG for the remainder of the study, using standard techniques (Ivens et al., 1978). A total of 933 fecal samples and 259 coprocultures were evaluated during the study.

Treatments

Several anthelmintics were used for treatment in the study; moxidectin (MOX; Quest®, Pfizer), ivermectin (IVER; Zimectrin®, Merial), generic ivermectin (GIVER; IverCare® Farnam), ivermectin with praziquantel (IVER-PRA; Zimectrin Gold®, Merial), fenbendazole (FEN; Safeguard®, Intervet), daily pyrantel tartrate (MOX-PYR; Strongid C 2X®, Pfizer), which was preceded by moxidectin according to manufacturer instructions, and pyrantel pamoate (PYR PAM; Strongid®, Pfizer). All dosages were given according to label dose rates and horse weight as determined with calibrated equine weight tape measurement at the heart girth. Treatments were given only to horses with a FEC >200 EPG. Owners and/or farm managers chose the anthelmintic at each treatment and were given the option to change treatments should their choice be ineffective (<90% FECR) at any point in the study.

Statistical analysis

Statistical analysis was performed using SAS for repeated measures (PROC MIXED, SAS Inst. Inc., Cary, NC) as described by Littell et al., 1996. Egg counts were transformed to the log 10(x + 1) prior

to analysis and significant differences were determined when the model *F*-test proved significant ($p < 0.05$).

IV. Results

Fecal Egg Counts

Of the 933 fecals examined during the study, 303 had EPG of zero, 407 were <200 EPG, and 223 were >200 EPG. In the group of horses sampled for all three phases, 126 samples were analyzed, with 37 samples with an EPG of zero, 58 samples <200 EPG, and 31 samples >200 EPG. In the group of horses sampled for only two phases, there were 550 samples analyzed, with 187 with an EPG of zero, 234 samples <200 EPG, and 129 samples >200 EPG. For horses sampled for only one phase, 256 samples were analyzed, with 79 that had an EPG value of zero, 115 samples <200 EPG, and 61 samples >200 EPG. There were 37 samples that contained eggs other than Strongyle-type eggs, including five with *Oxyuris equi*, two with *Parascaris equorum*, and 35 with cestode eggs (Figure 1).

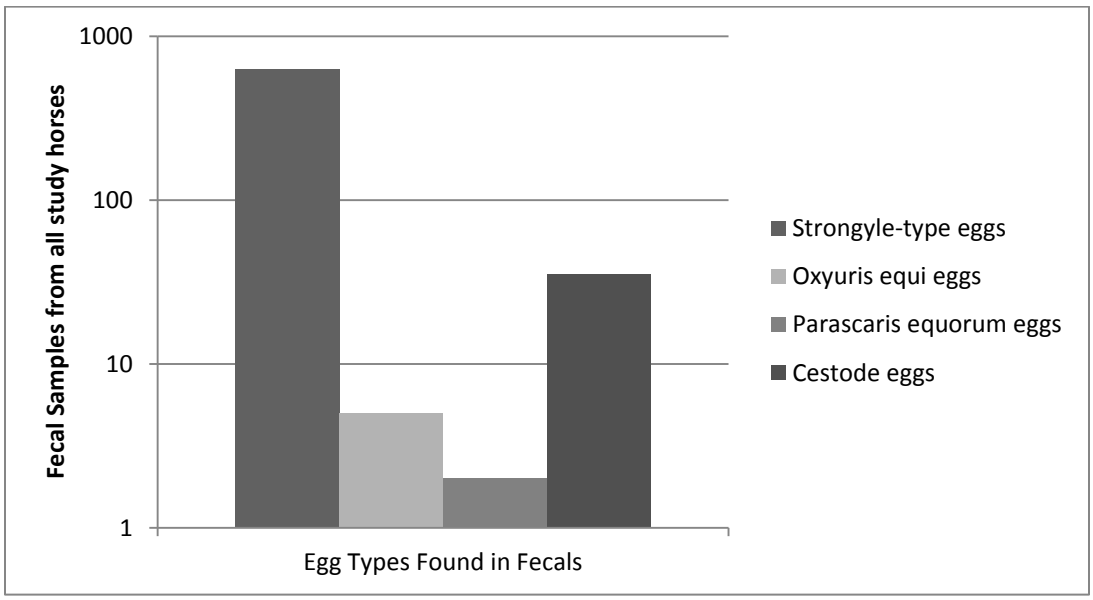

Figure 1. In 933 fecal samples collected from 227 horses over 8 months, 630 samples had Strongyle-type eggs, 5 samples had *Oxyuris equi* eggs, 2 samples had *Parascaris equorum* eggs, and 35 samples had cestode eggs. Data presented on a logarithmic scale.

Coprocultures

All three major large strongyle species were found in samples from horses in Northwest Arkansas. Seven of the 259 coprocultures had large strongyles, with one of the samples containing *S. vulgaris*, five with *S. equinus*, and two with *S. edentatus.* One sample had both *S. vulgaris* and *S. equinus*. The other 252 coprocultures contained only cyathastome larvae.

Treatments

A total of 156 treatments were given during the study. Of these, 107 treatments were MOX, 23 were ivermectin (10 IVER, 8 GIVER, and 5 IVER-PRA), 13 were FEN, and 13 were pyrantel (MOX-PYR, PYR, PYR-PAM) (Figure 2). Over the entire study, 101 horses did not require treatments, correlating to 44.5% of the animals used in the study.

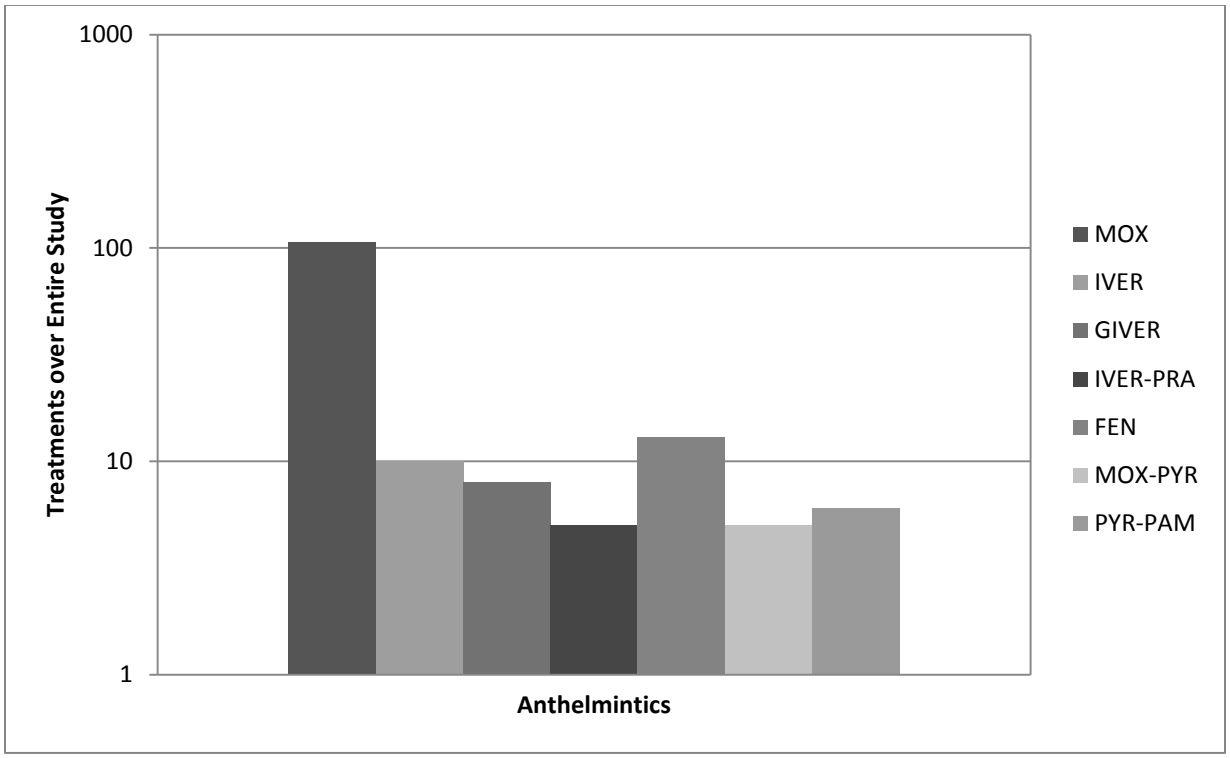

Figure 2. Out of 156 treatments given during an eight month selective deworming study, 107 were MOX, 23 were ivermectin (10 IVER, 8 GIVER, and 5 IVER-PRA), 13 were FEN, and 13 were pyrantel (2 MOX-PYR, 6 PYR-PAM).

In the horses sampled for three phases, six of the horses were considered CON (untreated group) animals (0 EPG or <200 EPG) throughout the study, five horses were treated one time (FEC >200 EPG), 10 horses were treated twice, and there were no horses that needed to be treated at all three

phases. Two of the horses treated once were given MOX during the first phase and were in the CON group for the other two phases. Two other horses were in the CON group for the first two phases, then treated with MOX in the third phase, while the last horse was in the CON group for the first and third phase, and treated with GIVER during the second phase. Of the 10 horses requiring two treatments, 8 were treated initially with MOX, then had EPG lower than the threshold in the second phase, then required treatment again in the third phase. One of these horses was treated with GIVER and the others were all treated with MOX. In two other horses that required two treatments, the initial fecal sample put them in the CON group but they were treated the remaining two times with MOX.

The horses sampled for two phases consisted of 50 CON animals, 44 that were treated once, and 22 that were treated twice. Of the 44 horses treated once, 32 were treated with MOX, six were treated with IVER, three were treated with FEN, two were treated with MOX-PYR, and one was treated with IVER-PRA. Of the 22 horses treated twice, eight were treated with MOX both times, four were treated initially with GIVER and then MOX, four were treated initially with FEN and then IVER, two were treated with FEN and then PYR-PAM, one was initially treated with FEN and then MOX, one was treated initially with FEN and then IVER-PRA. Horses sampled for one phase included 45 CON animals and 44 treated horses. Of the treated horses, 21 were treated with MOX, five were treated with IVER, two were treated with FEN, and two were given PYR-PAM.

Drug Efficacy

Efficacies were determined for MOX during the first phase of treatments, and MOX and IVER during the second phase of treatments. During the first phase of treatments, 134 CON animals had a PRT FEC average of 43.9 EPG (arithmetic mean-AM) and a PT average FEC of 96.9 EPG (AM), resulting in a FECR of +177.7% (based on AM). The MOX treated animals (N = 56) had a PRT average FEC of 768.8 EPG (AM) and a PT average FEC of 13.8 EPG (AM), resulting in a FECR of 98.2% (based on AM). The PRT average and PT average were statistically different ($P<0.05$) for CON and MOX animals in the first phase of the study (Figure 3).

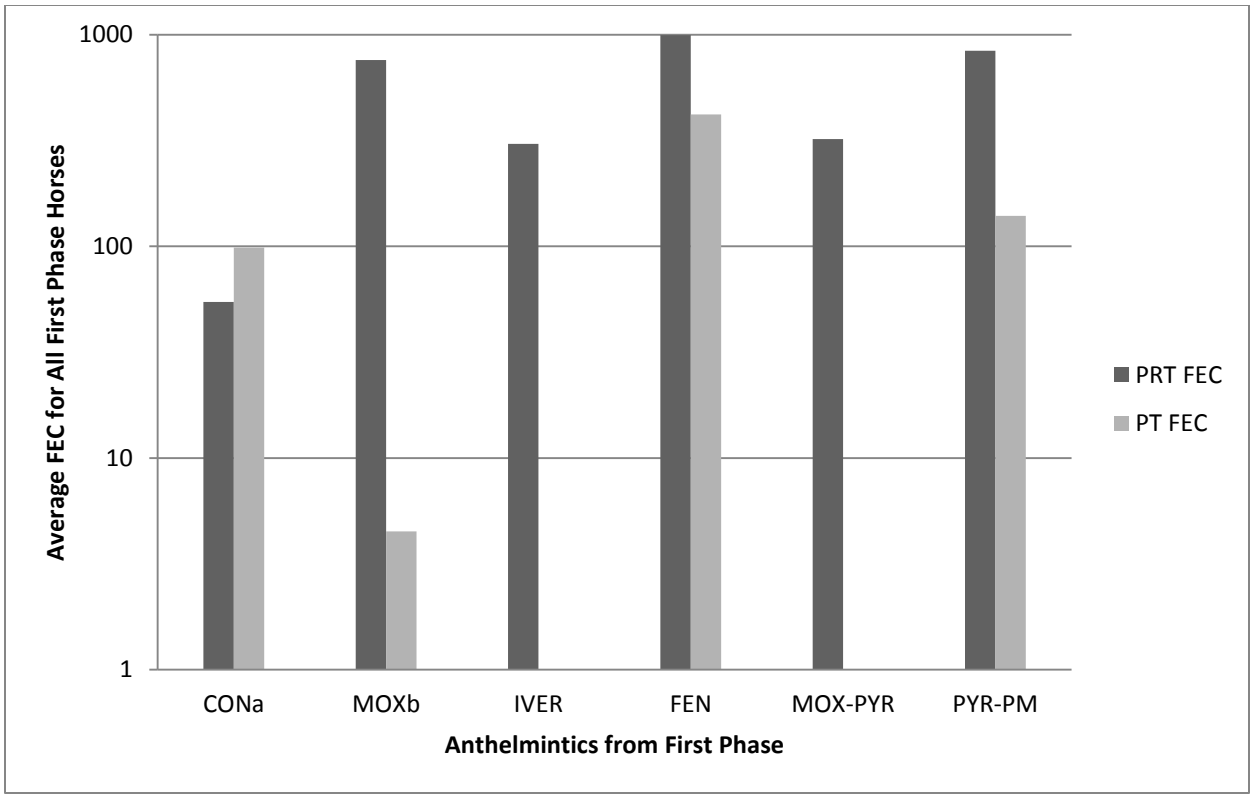

Figure 3. First phase treatment groups with pre-treatment (PRT) and post-treatment (PT) fecal egg counts (FEC) for each group. All FEC are averages for all animals in each treatment group. CON animals (N=137) had PRT FEC of 54.6 EPG and PT FEC of 98.7 EPG; MOX animals (N=53) had PRT FEC of 759.4 EPG and PT FEC of 4.5 EPG; IVER animals (N=11) had PRT FEC of 304.3 EPG and PT FEC of 0 EPG; FEN animals (N=13) had PRT FEC of 994.6 EPG and PT FEC of 420 EPG; MOX-PYR animals (N=4) had PRT FEC of 321.3 EPG and PT FEC of 0 EPG; PYR-PAM animals (N=2) had PRT FEC of 838.5 EPG and PT FEC of 139 EPG. CON and MOX were statistically different ($P<0.05$) for PRT FEC and PT FEC, as indicated by a,b subscripts. Data presented on logarithmic scale.

During the second phase of treatments, 103 CON animals had a PRT average FEC of 32.3 EPG (AM) and a PT average FEC of 110.2 EPG (AM), resulting in a FECR of +241.2 % (based on AM). The MOX treated animals (N = 30) had a PRT FEC average of 434.1 (AM) and a PT average FEC of 10.7 EPG (AM), resulting in a FECR of 97.5% (based on AM). The IVER treated animals (N = 12) had a PRT FEC average of 334.6 EPG (AM) and a PT average FEC of 2.1 EPG (AM), resulting in a FECR of 99.3% (based on AM). The PRT and PT average FEC of the MOX and IVER treated horses were not different than each other, but both were statistically different than the CON animals ($P<0.05$) (Figure 4). During the third phase of treatments, there were 34 CON horses with an average PRT FEC of 31 EPG and a PT average FEC of 142 EPG, resulting in a FECR of +358%. All treated horses in the third phase were

treated with MOX (N = 27) with an average PRT FEC of 955 EPG and an average PT FEC of 0.1 EPG, with a FECR of 99.9% (Figure 5).

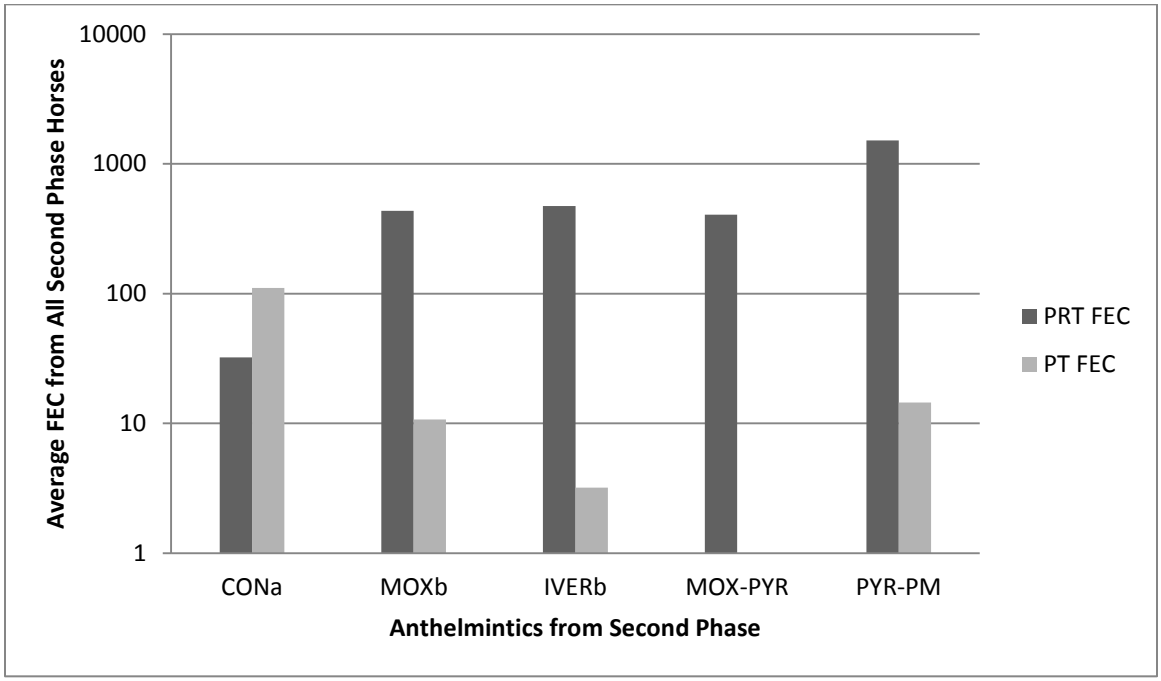

Figure 4. Second phase treatment groups with pre-treatment (PRT) and post-treatment (PT) fecal egg counts (FEC) for each group. All FEC are averages for all animals in each treatment group. CON animals (N=103) had PRT FEC of 32.3 EPG and PT FEC of 110.2 EPG; MOX animals (N=30) had PRT FEC of 434.1 EPG and PT FEC of 10.7 EPG; IVER animals (N=12) had PRT FEC of 471.7 EPG and PT FEC of 3.2 EPG; MOX-PYR animals (N=2) had PRT FEC of 405 EPG and PT FEC of 0 EPG; PYR-PAM animals (N=2) had PRT FEC of 1510 EPG and PT FEC of 14.5 EPG. No horses were treated with FEN in the second phase. MOX and IVER were statistically different from CON ($P<0.05$) for both PRT FEC and PT FEC, as indicated by a,b subscripts. Data presented on logarithmic scale.

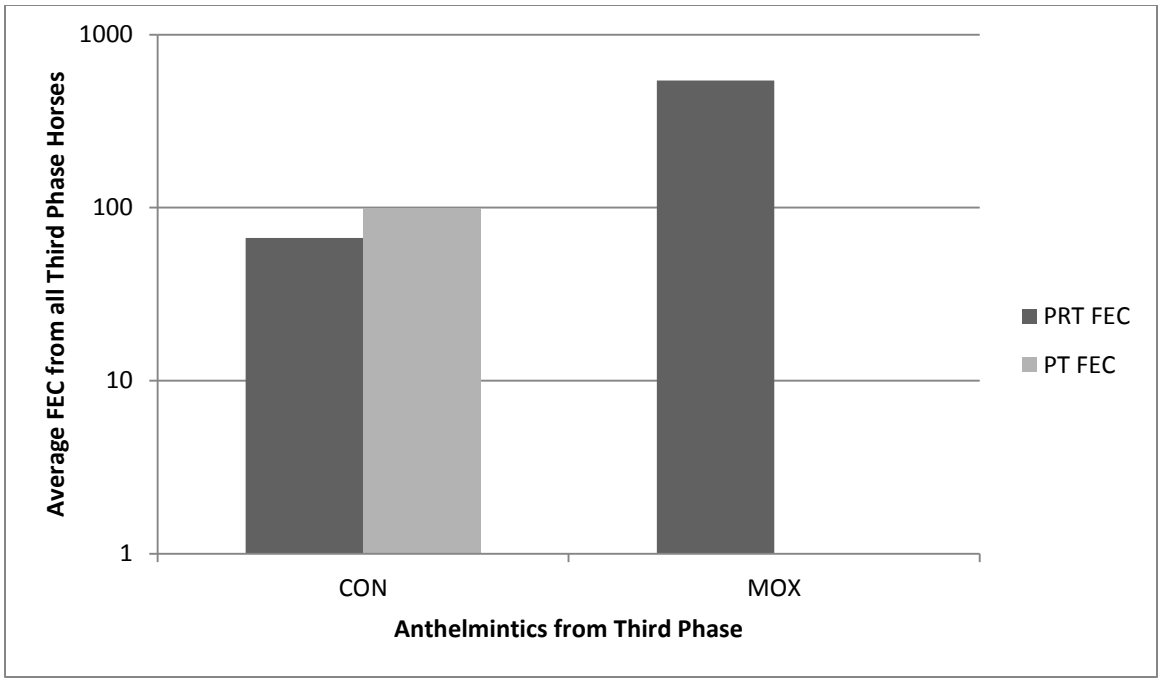

Figure 5. Third phase treatment groups with pre-treatment (PRT) and post-treatment (PT) fecal egg counts (FEC) for each group. All FEC are averages for all animals in each treatment group. CON animals (N=9) had PRT FEC of 66.7 EPG and PT FEC of 99.2 EPG; MOX animals (N=12) had PRT FEC of 542.2 EPG and PT FEC of 0.1 EPG. No horses were treated with IVER, FEN, MOX-PYR, or PYR-PAM during the third phase. Data presented on logarithmic scale.

A total of 19 horses were treated with drugs other than MOX and IVER during the study. Thirteen horses were given FEN, with an average PRT FEC of 995 EPG and an average PT FEC of 420 EPG. Based on these averages, the FECR of FEN was 57.7 % during the study (Figure 3). There were four horses given pyrantel pamoate, with an average PRT FEC of 1174 and a PT average FEC of 77 EPG, resulting in a FECR of 93.4% (Figure 3; Figure 4). Seven horses were treated with MOX-PYR, with an average PRT FEC of 299 EPG and an average PT FEC of 0 EPG, resulting in a FECR of 100% (Figure 3; Figure 4).

V. Discussion

The fecal egg count data presented here is consistent with similar studies. A major problem with relying on fecal egg counts for a selective treatment protocol is that the diagnostic test primarily targets only strongyle parasites, primarily cyathostomins. Although these are the most abundant gastrointestinal parasites in adult horses, other parasites can be implicated in disease and should be monitored (Kaplan

and Nielsen, 2010; Nielsen et al, 2014). In this study, only 37/933 samples (3.9%) contained eggs from parasites other than strongyles with the most common being cestode eggs. This suggests that the prevalence of gastrointestinal parasites other than small strongyles is low enough to not warrant frequent treatment, which has been previously suggested (Kaplan and Nielsen, 2010). The number of samples with *P. equorum* eggs, another important parasite, was very low (N=2) but the majority of horses sampled in this study were at least one year of age; a "cut-off" age for when clinical infections with this parasite become quite rare (Nielsen et al, 2013).

Although indirect monitoring of large strongyle infections does occur with fecal egg counts, their eggs cannot be differentiated from those of cyathostomins and coprological techniques must be used; a technique that requires a certain amount of proficiency (Nielsen et al, 2014). A survey of Danish veterinarians reported 41% of respondents used coprocultures in their practice and most perceived that large strongyles rarely caused problems (Nielsen et al, 2006). In the current study, coprocultures were only done on samples that had fecal egg counts greater than 20-50 EPG. The prevalence of all three major species of large strongyles was very low, with only 7/259 cultures positive for at least one species (2.7%). The major pathogenic nematode, *S. vulgaris,* was only identified in one sample. This low prevalence has been found in another study using PCR-screenings (Nielsen et al, 2012b) and as few as one to two yearly larvicidal treatments are expected to adequately control *S. vulgaris* infections in most adult horses (Nielsen et al, 2012a).

The treatment frequency results from this study are consistent with other selective deworming studies, wherein not all horses need to be dewormed regularly, based on fecal egg counts (Becher et al, 2010; Kaplan and Nielsen, 2010, Larsen et al, 2011). During this study, 44.5% of the horses did not exceed the threshold for treatment (>200 EPG); leaving about one-half of the horses untreated. Since it is common for all horses to be treated on a regular basis, such as every three months with interval deworming, using fecal egg counts to determine treatment could reduce the number of treatments given on a specific farm, as well as to individual horses; which is desirable in terms of economics and expanded refugia.

Efficacy data in this study is consistent with current studies, although there is little published data concerning anthelmintic efficacy with selective treatment protocols. Fenbendazole in this study had a low sample size (N=13) and a FECR of 57.7%. Resistance of fenbendazole is well documented in the U.S. and Europe (Varady et al., 2000; Chandler and Love, 2002; Varady et al., 2004; Rossano et al., 2010), which could account for the owner's decisions to use other products in this study. The efficacy of pyrantel products in this study is difficult to elucidate since there were very few horses on these products, and the manufacturer recommendations for pyrantel tartrate include pre-treatment with moxidectin. It is unclear if low egg counts were due to the pyrantel products or moxidectin. Ivermectin efficacy in this study was very high, with an average FECR of 99.7%. This is consistent with recent studies evaluating ivermectin efficacy in the face of concerns with macrocyclic lactone resistance (Klei et al, 2001; Larsen et al., 2011). The efficacy of moxidectin was also high, with an average FECR of 98.5%, and potential contribution to the low FEC in the pyrantel treated horses. Repeated studies have shown consistently high efficacies of moxidectin, although many legitimate concerns have been raised regarding its prolonged residues in tissue leading to possible resistance to this drug in the future (Kaplan, 2002; Rossano et al., 2010).

VI. Conclusion

The results of this study, combined with information from other published studies and the AAEP, offer some guidance for anthelmintic treatment of horses using selective treatment protocols. Fecal egg counts should be integrated into regular herd health protocols, particularly those with adult horses, in order to reduce egg shedding and treatment rates, which could reduce anthelmintic resistance (Kaplan and Nielsen, 2010). For veterinarians, fecal egg counts should be a part of regular practice and a strong recommendation to clients for proper management of equine health. While current research is making strides in managing equine gastrointestinal parasites, more research is needed in the field. Extensive field surveys of treatment rates, egg shedding rates, and anthelmintic efficacy have not been reported, and would greatly impact reception and implementation of the current AAEP recommendations (Nielsen, 2012c).

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VIII. Appendix

a. PARASITE EGGS AND LARVAE

Figure 6. Strongyle-type egg in equine fecal sample at 400x magnification. Photo by Dr. Chris Tucker and used with permission.

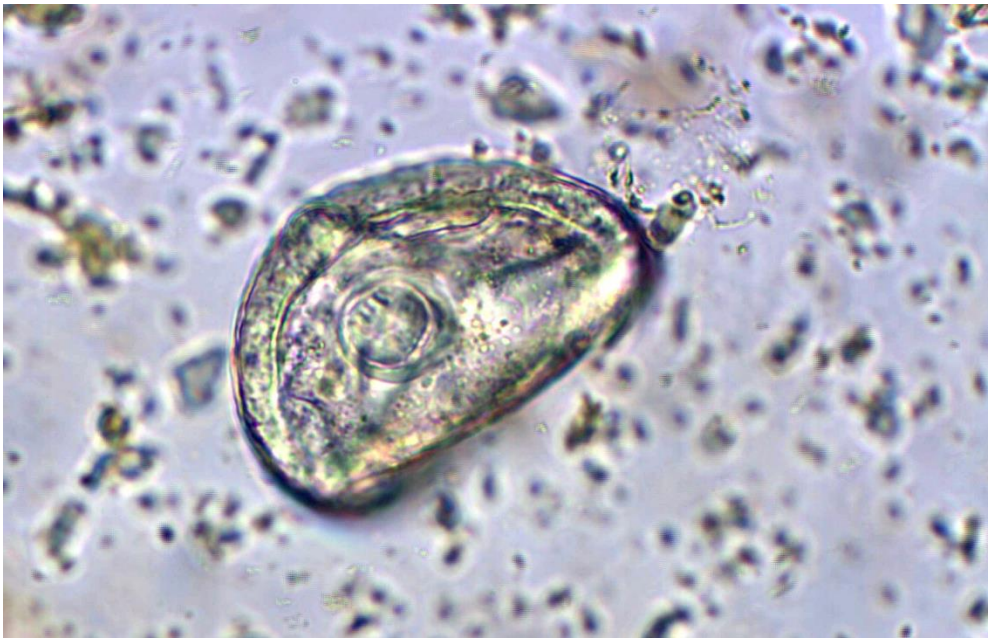

Figure 7. Cestode (*Anaplocephala spp.*) egg in equine fecal sample at 400x magnification. Photo by Stephanie Rainbolt and used with permission.

Figure 8. Pinworm (*Oxyuris equi*) egg in equine fecal sample at 400x magnification. Photo by Stephanie Rainbolt and used with permission.

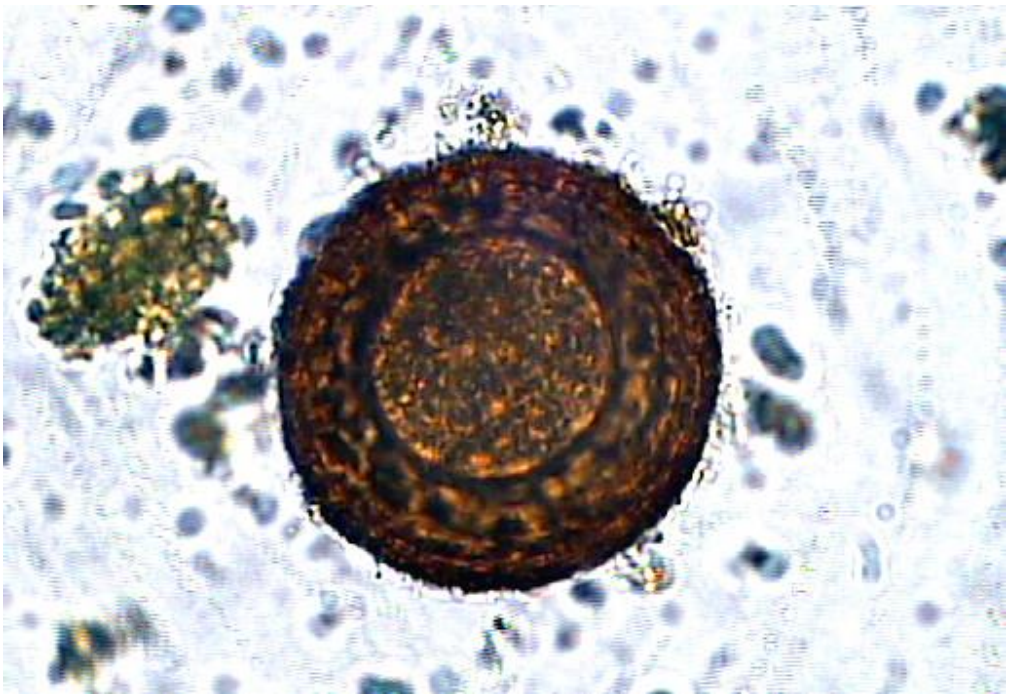

Figure 9. Ascarid (*Parascaris equorum)* egg in equine fecal sample at 400x magnification. Photo by Dr. Chris Tucker and used with permission.

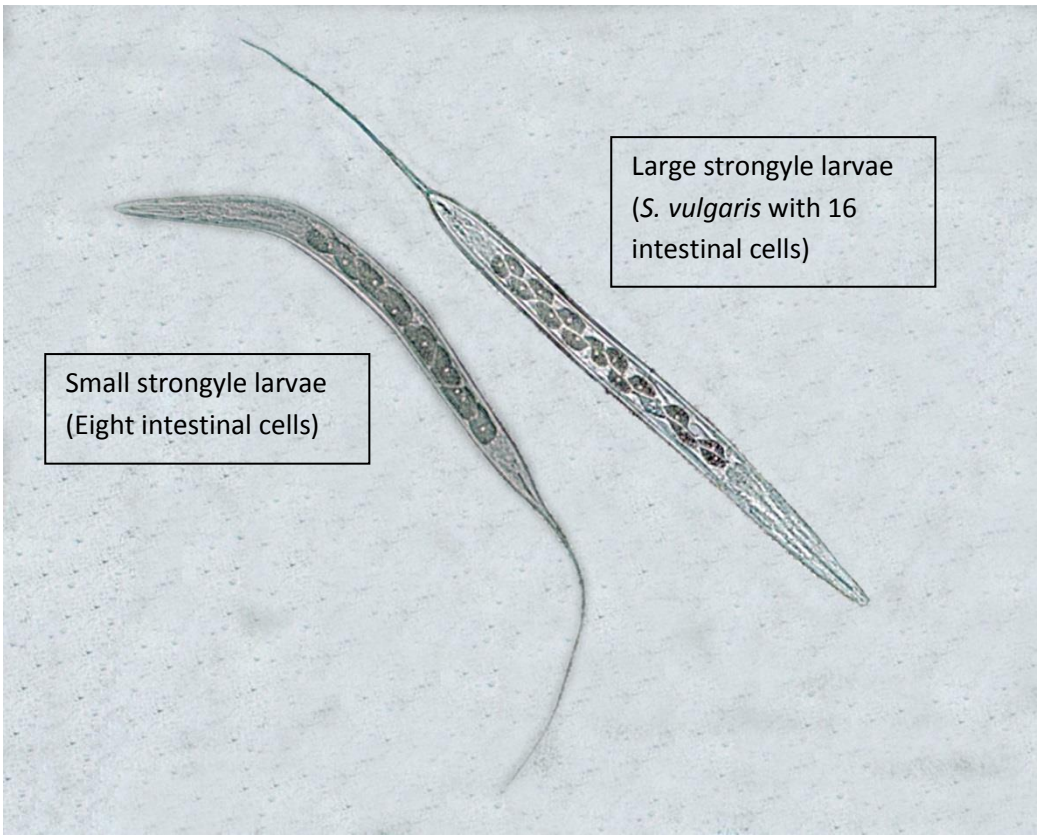

Figure 10. Small strongyle and large strongyle(*Strongylus vulgaris*) L1 larvae at 100x magnification. Photo by Stephanie Rainbolt and used with permission.

b. IACUC EXEMPTION

This project only involved the collection of fecal samples so approval from the Institutional Animal Care and Use Committee (IACUC) was not needed. The IACUC is concerned with animal welfare in studies where animals or their environment are manipulated in some fashion, or where study activities such as methods of observation cause excessive disturbance to animal behavior. Work with naturally occurring samples, animal carcasses, or observation of animals in their natural settings are most often exempt.

Tony Munn CMAR LATG
IACUC ADMINISTRATOR
A42 AFLS
1120 West Maple St.
Fayetteville, AR 72701
Ph # (479) 575-2994