INVESTIGATION OF ANTHELMINTIC RESISTANCE AND
DE-WORMING REGIMENS IN HORSES

by

MEGHAN BLANEK, B.S.

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Approved

Adelaide Anne Brady
Chairperson of the Committee

Kevin R. Pond

Samuel Paul Jackson

David Bruce Wester

Accepted

John Borrelli
Dean of the Graduate School

August, 2005

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ABSTRACT

Parasite resistance has emerged as a health dilemma in the livestock industry as many classes of parasites are becoming resistant to dewormers. Parasitism is very costly to the equine industry as it affects the performance and health of horses. Resistance has been documented for every type of equine anthelmintic class. A series of three studies was conducted to determine the efficacy of different deworming regimens. In Study 1, three fenbendazole deworming regimens were given to assess efficacy as determined by fecal egg reduction and to test for possible anthelmintic resistance in a herd that was administered only fenbendazole for 18 months prior. Resistance to fenbendazole was documented in this population based on fecal egg count reduction (FECR) tests and DrenchRite assays. Significant differences in FECR were observed between the larvicidal treatment and 5 mg/kg treatment on both day 42 versus 28 ($P < 0.05$) and day 72 versus 56 ($P = 0.10$). When analyzed by age of young horses (< 2 years of age) versus mature mares (> 2 years of age), there was a significant difference on both day 42 versus 28 and day 72 versus 56 ($P < 0.05$). In Study two, the efficacy of moxidectin, ivermectrin, and the larvicidal dose of fenbendazole were studied in this herd of known resistance. Mean FECR tested on day 14 post treatment was 99.91%, 98.73%, and 89.07% respectively, for the three treatment groups. Moxidectin and ivermectin groups were significantly different from the fenbendazole group ($P < 0.05$). When analyzed by age however, there were no differences between treatment groups in the mature mares (> 2 years of age). However, the moxidectin and ivermectin were significantly different from the fenbendazole group in the young horses (< 2 years of age) ($P < 0.05$). Study

three determined the efficacy of a year-long rotation on breaking fenbendazole resistance in a field setting. A farm wide study showed a quarterly rotation of pyrantel pamoate (June), ivermectin (September), a larvicidal dose of fenbendazole (December), and moxidectin (March) broke resistance based on fecal egg count reduction tests. The mean fecal egg count reductions for the treatments, tested on day 7 post treatment, were 95.86%, 100%, 97.84%, and 100% respectively.

KEYWORDS: Parasite, Anthelmintic, Resistance

CHAPTER I

INTRODUCTION

Anthelmintic resistance has emerged as a health dilemma in the livestock industry as many parasites are becoming resistant to dewormers. Anthelmintic resistance was first reported in the early 1960's (Craig, 1993). As large strongyles intestinal parasites are being effectively controlled by routine use of anthelmintics, a new problem concerning resistance in small strongyles (cyathostomes) has been widely documented world wide (Barger, 1974; Dorny et al., 1988; French and Klei, 1983). The cyathostomes are the most difficult to treat due to the fact that they become encysted in the gut wall and are associated with general resistance. The mechanisms of parasite resistance to current equine anthelmintics have been reviewed (Sangster, 1999). Some signs of parasitism are diarrhea, rapid weight loss, loss of body condition, marked weakness, colic, and death in severe cases. The common classes of equine anthelmintics include benzimidazoles, tetrahydropyrimidines, and avermectin/milbemycins. Reports of resistance to benzimidazoles in the literature have been associated with the frequent administration of benzimidazole products to a herd for a significant period without rotation of different classes of anthelmintics or with frequent dosing intervals. This research involved a three-part study on the efficacy of anthelmintics in a herd of horses administered only fenbendazole for 18 months prior.

CHAPTER II

LITERATURE REVIEW

Parasitism in Horses

Parasitism in horses can result in substantial health problems, decreased performance, and death. Parasitism is a very common disease of horses today and is one of the most costly and damaging equine diseases. The economic impact of this problem can be significant if not effectively controlled. Severe cases of parasitism are very costly to horse owners and breeders alike. Effective control of parasites should be a major priority in quality horse management programs. Misuse of equine anthelmintics, poor management strategies, and poor understanding of this aspect of horse health care, however, are prevalent throughout the horse industry. The clinical signs of parasitism in horses include weight loss, poor feed efficiency, dull hair coat, depression, low energy, colic, and diarrhea.

Anthelmintic resistance has emerged as a dilemma in the livestock industry as many parasites are becoming resistant to dewormers. Anthelmintic resistance was first reported in the early 1960's (Craig, 1993). Although many formerly life threatening intestinal parasites are being effectively controlled by the routine use of anthelmintics, new problems are emerging as increased resistance to anthelmintics is being reported worldwide.

Types of Equine Parasites

Ascarids

Ascarids, also known as the large roundworm and scientifically known as *Parasacaris equorum*, are only found in horses. The ascarid, the largest parasite in the horse, is yellowish-white in color, and can grow up to 12 to 15 inches long. They live in the small intestine where they mate and produce eggs that are passed out through the feces. Within two weeks, the eggs become infective and are ingested by other horses. Ascarids can live for years in stalls, paddocks, and pastures. Once the eggs have been ingested, the larvae hatch in the intestine where they penetrate the small mesenteric blood vessels. After entering the blood stream, they migrate to the liver and the lungs. The worms then crawl or are coughed up to the trachea, are re-swallowed, and return to the intestine where they undergo maturation. Ascarids can cause severe damage to the liver and lungs through migration. In young horses, intestinal blockage can occur with large numbers of adult ascarids present, which can also lead to rupture of the intestine. The common symptoms of ascarid infection are a dull, rough hair coat, potbellied appearance, and poor growth or unthriftiness. Treatment of ascarids is recommended for the young foal, starting at two months of age. Ascarids are not a common problem in the mature horse, however, due to the development of immunity. (Evans, 2001)

Pinworms

The pinworm is the common name for *Oxyuris equi*. The pinworm is white in color and can grow up to 3 inches long. The worms live in the colon and survive on the gut contents. The eggs are passed through the feces, become mixed with grass, water,

and feed, and are ingested by the horse. In a period of several weeks, the eggs hatch into larvae, and mature into adults in the intestine. Pinworms, however, does not penetrate the gut wall. Once the females have mated in the colon, they migrate to the rectum where most of the females lay eggs, which are expelled in the feces. However, some of the females crawl to the anal opening and lay eggs on the perianal region of the horse, causing irritation to this area. As the horse rubs this region on posts or barns, the eggs drop to the ground. (Evans, 2001)

Strongyles

Strongyles are described into two main classes, large strongyles and small strongyles.

Large Strongyles

Three species of large strongyles occur in the horse: *Strongylus edentatus*, *Strongylus equines*, and *Strongylus vulgaris*. Once the eggs are passed through the feces, the eggs hatch within one day under favorable conditions. Within the next week, they molt to the third stage larvae in which they are capable of climbing the blades of the grass then are ingested by the horse. (Evans, 2001)

Strongylus edentatus is classified as the medium-sized toothless strongyle. They are approximately 1½ inches long and usually found in the ventral colon and cecum. Once ingested, they penetrate the mucosa of the intestines and migrate extensively throughout the abdominal cavity and organs. The larvae continue to migrate while

developing to mature adults when after have returning to the intestinal tract. The adult worms attach to the intestinal mucosa and suck blood from the host. (Evans, 2001)

Strongylus equines is often called the large-toothed strongyles. They grow up to two inches long and are found in the ventral colon and cecum. Once ingested by the horse, they burrow through the intestinal wall, migrate though the liver and pancreas and return to the intestines. These parasites are very rare and only found in the western portion of the U.S. (Evans, 2001)

Strongylus vulgaris is one of the most harmful internal parasites in the horse. Once the larvae are ingested, they penetrate the intestinal mucosa. The larvae migrate to the small blood vessels producing scar tissue as they migrate, which causes the most damage. The irritation and inflammation caused by the larvae results in formation of thrombosis, embolism, and a weakening of the arterial walls. Colic or death can result in migration through or blockage of the anterior mesentery artery. The adult worms attach to the mucosa of the cecum and colon and suck blood. (Evans, 2001)

Small Strongyles

Small strongyles (cyathostomes) are the common name for a group of about 40 to 50 scientific species of equine pararsites. They vary from grayish-white to pink in color and are less than ¼ inch and up to 1 inch in length. These nematodes are very common in horses. Today, parasitologists consider encysted cyathostomes to be the #1 nematode problem of horses (Intervet, 2000). The small strongyles can account for up to 90% of the horse's total parasite burden. Small strongyles have five different stages in their life cycle. The adult small strongyles lay eggs in the colon which are passed out through the

feces to the pasture, where the eggs hatch (pre-parasitic stage). The hatched eggs develop into third stage larvae (L_3) which are ingested with the forage. The second phase is the parasitic phase in which third stage larvae (L_3) enter into the small intestine and migrate to the cecum and colon. The encysted phase is the third stage of the small strongyle life cycle. Once the L_3 penetrate the intestinal lining and become encysted, they become early third stage larvae (EL_3). Many encysted EL_3 's become hypobiotic, which can last up to three years. Encysted cyathostomes are hard to control as many anthelmintics are ineffective against this stage. Some EL_3 's will evolve into late third stage (LL_3) and then molt to the fourth stage (L_4). The L_4 will encyst and emerge into the gut lumen where they will molt on to the fifth stage larvae (L_5). After this stage they develop into adults and lay eggs that are passed out through the feces.

Larval cyathostomosis results when fourth-stage larvae synchronously emerge from mucosal cysts in the cecum and colon. "The typical acute clinical syndrome associated with larval cyathostomosis consists of profuse, sudden onset diarrhea, which may be persistent or intermittent; severe, rapid weight loss with a normal appetite and water intake; loss of body condition, with muscle wasting in severe cases; marked weakness but possible lack of depression; and subcutaneous edema of the limbs and ventral abdomen" (Paul, 1998). Death can occur and be relatively sudden in severe cases of parasitism. Larval emergence causes mechanical damage, but also releases waste materials sequestered within the cyst, resulting in focal or general typhlitis and colitis. Large strongyle infection result when the parasites emerge at once and cause intestinal blockage resulting in colic. Cyathostomosis is common in England, however, it is not as common in America.

Bots

Bots, also known as *Gasterophilus spp.* larvae, are the larval or maggot stages of bot flies. They are also referred to as stomach bots as they are found in the stomach. The horse is the only host of the botfly. The adult botfly lays its eggs on the hairs of the legs, throat, and chin of the horse. The eggs hatch only from the stimulus warmth and moisture from the horse licking or biting the area in which the eggs were deposited. The larvae are taken in internally through the horses' mouth and can remain in the horse for 8-10 months. Once inside the horses' mouth, the larvae penetrate the mucous membranes and migrate to the stomach, attaching to the lining of the stomach wall. In early spring, they detach themselves, pass out through the feces and burrow into the ground before maturation into adults. (Evans, 2001)

Tapeworms

The tapeworm, also known as *Anoplocephala*, infest horses, but the horse usually will not show clinical symptoms. In the horse, tapeworms are found in the cecum and lower part of the small intestine. They are about 1-inch long. These tapeworms are transmitted by oribatid mites, who ingest the eggs and the mites are ingested during grazing by the horse. (Evans, 2001)

Other Parasites

Other less common equine parasites include: threadworms, small stomach worms, neck threadworm, and lungworms. Threadworms are found in the small intestines of foals and can be passed through the mare's milk. It is believed that horses become

immune to these parasites. Small stomach worms are the larvae of houseflies or stable flies. Internally, they can cause chronic gastritis and externally they can migrate into open skin wounds and can prevent healing. The neck threadworm lives in the Ligamentum nuchae of the horse. This parasite produces larvae called microfilariae which migrate through the horse to the ventral abdomen or the tip of the head. The microfilaria has been recovered from the eyes which can cause moon blindness. Lungworms are common in donkeys, in which they live in the lungs and cause chronic coughing and respiratory distress. (Evans, 2001)

History of Equine Anthelmintics

Anthelmintics are the common solution to controlling nematodes in current equine management practices. Historically, one of the first medications used to treat parasites was oil of chenopodium which was distilled from the seeds or leafy part of *Chenopodium anthelminticum* (Lyons et al., 1999). This was also used by the early settlers as a treatment for ascarids in humans. Other early types of anthelmintics were hens' eggs, guts of the young hen and pigeon, dung from hens and man, black soap, calomel, aniseed, aloes, antimony, liquorice, linseed, and vitriolated quicksilver (Lyons et al., 1999). The administration of many of these compounds risked toxicity to the horse and was not effective in removing parasites. Carbon disulfide was used in the early 1900's. Phenothiazine, emerging in the 1940's, was the next drug class used to treat nematodes and was used for the control of parasites for 20 years. In the 1950's, the class of piperazines was introduced. The benzimidazoles emerged on the equine market in the 1960's and 1970's, the organophosphates emerged in the 1960's (trichlorfon) and 1970's

(dichlorvos), and levamisole and pyrantels were introduced in the 1970's. The class of macrocyclic lactones were approved for equine use in the 1980's (ivermectin) and 1990's (moxidectin) (Lyons et al., 1999). (See Figure 1)

Phenothiazine was found to be active against strongyles and was the most commonly used for twenty years. The drug was also used as a daily top dressing on the grain ration as it would block or reduce transmission by affecting reproduction of the parasites. This method was used from the 1940's to 1950's (Lyons et al., 1999).

Piperazine is a gamma-amino butyric acid agonist that causes spastic paralysis of the parasite by hyperpolarizing the muscle membrane potential and increasing membrane conductance (Love, 2003). Adipate, hydrochloride, citrate, and phosphate are the different piperazine salts used. This class was generally used with a mixture of another compound for broader spectrum activity against nematodes. Piperazines were found effective against ascarids, strongyles, and pinworms. This drug, however, was very effective against the removal of small strongyles while removal of large strongyles was very poor. Piperazine has been mixed with carbon disulfide, phenothiazine, thiabendazole, levamisole, and trichlorfon.

Trichlorfon was the first organophosphate to be marketed and was effective against bots, ascarids, and mature pinworms. As it was not effective against strongyles, it was mixed with phenothiazine, piperazine, mebendazole, oxfendazole, and thiabendazole. Dichlorvos was the second organophosphate used. The pelleted form of dichlorvos that was active against small strongyles, bots, ascarids, large strongyles, and pinworms, however, was toxic to birds if eaten. The gel formulation was effective against bots, ascarids, and pinworms.

Figure 1. Chronology of marketing of anti-parasitic drugs for equids in the USA. CS2: Carbon Disulfide; PTZ: phenothiazine; PPZ: Piperazines; TCF: Trichlorfon; BZs: Benzimidazoles; DDVP: Dichlorvos; PRT: Pyrantels; LEV: Levamisole; IVE: Ivermectin; MOX: Moxidectin. (Adapted from Lyons et al., 1999)

Currently there are three classes of dewormers commonly used in the equine industry: benzimidazole, tetrahydropyrimidines, and avermectin/milbemycins. Also, there is piperazine, the fourth drug class that is rarely used. Fenbendazole, oxfendazole, oxibendazole, ivermectin, moxidectin, piperazines, pyrantel pamoate, and pyrantel tartrate are the compounds currently used on the market in the United States (Lyons et al., 1999).

Benzimidazoles

Thiabendazole was the first drug of the benzimidazole class to be developed in the early 1960's (Lyons et al., 1999). Later cambendazole, fenbendazole, mebendazole, oxfendazole, and oxibendazole were introduced. When first marketed, these anthelmintics had excellent activity on the major nematodes while having low dose rates and low toxicity.

The chemical name of fenbendazole is methyl 5-(phenylthio)-2-benzimidazole carbamate. The primary effect of the group of benzimidazoles is that of binding tubulin to prevent polymerization or to cause depolarization of the microtubules in intestinal cells of helminthes, which causes death to the parasite by starvation (Craig, 1993). Higher doses or prolonged treatment may bind to heterozygote tubulin, however, it increases the rapid selection of the recessive benzimidazole resistant trait (Craig, 1993). This group is primarily effective against cyathostomes (EL₃, LL₃, and L₄ stages), large strongles, ascarids, and pinworms. However, the larvicidal treatment of fenbendazole is very effective against cyathostomes (EL₃).

Tetrahydropyrimidines

Pyrantel pamoate is a compound belonging to the class of the tetrahydropyrimidines which was not developed until the 1970's. The chemical name for pyrantel pamoate is (E)-1, 4, 5, 6-tetrahydro-1-methyl-2-[2-(2-thienyl)-vinyl]-pyrimidine 4,4' methylenebis [3-hydroxy-2-naphthoate] (1:1). This drug causes spastic paralysis of parasites by selective agonist action at synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells (Love, 2003). This group is primarily affective against large and small strongyles, pinworms, and large roundworms and highly effective against tapeworms.

Avermectin/milbemycin

Macrocyclic lactones include both avermectins and milbemycins. Ivermectin, in the avermectin group, was first marketed in the 1980's (Lyons et al., 1999). This drug selectively paralyzes parasites by increasing muscle chloride permeability through interaction with glutamate-gated chloride ion channels (Love, 2003). Ivermectin is primarily effective against large strongyles, small strongyles, ascarids, pinworms, hair worms, and stomach bots.

Moxidectin, in the milbemycin group was first marketed in the 1990's (Lyons et al., 1999). This drug selectively paralyzes parasites by increasing muscle chloride permeability through interaction with glutamate-gated chloride ion channels (Love, 2003). Moxidectin has a much longer half-life in body fat than ivermectin as it is highly lipophilic, therefore it works slower than ivermectin. This drug can be toxic to young foals and emaciated animals with insufficient adipose tissue because of the drug's high

lipophilicity (Love, 2003). This group is primarily effective against large strongyles, encysted small strongyles, ascarids, pin worms, hair worms, and stomach bots.

Praziquantel, which is used in the horse in a combination product with ivermectin, is highly effective against tapeworms. This drug increases the permeability of tegumental and muscle cells to calcium, which results in parasitic muscular contraction and paralysis (Love, 2003).

Resistance

As defined by Sangster (1999), anthelmintic resistance is the ability of parasites in a population to survive treatments that are generally effective against the same species and stage of infection. Studies have shown that anthelmintic resistance is inherited, requiring resistant genes to be present (Sangster, 1999). When the horse is dewormed, the non-resistant worms are killed and passed out of the horse, allowing the resistant worms to have less competition and therefore, have a reproductive advantage. “Such intervals of reproductive advantage follow each use of a resistance-prone dewormer, so selection for resistance is intensified and accelerated by repeated and frequent treatments” (Reinemeyer, 2003) Resistance can also occur within classes known as side-resistance or between classes known as cross-resistance.

Several factors can influence the rate of development of resistance on farms. Studies in sheep have shown that reducing the frequency of treatment is the most important factor in reducing selection pressure for resistance (Sangster, 1999). Reducing the frequency of the treatment increases the likelihood of resistant survivors being diluted with susceptible ones. Weather is also crucial in resistance as weather conditions can

determine if survivors of the treatment will reproduce and seasonal patterns determine when encysted larvae emerge into the intestinal lumen (Sangster, 1999). Under-dosing of the drug, either by underestimation of the horse's weight or by loss of the anthelmintic during administration, can also influence the selection for resistance in parasites as it is like using an ineffective drug. Continued use of one class of anthelmintic without rotation is also associated with the development of resistance.

Many studies have defined resistance in several different ways. Coles et al. (1992) and the Federal Drug Administration define resistance as Fecal Egg Count Reductions (FECR) of <90% when reduction analysis is determined 14 days after treatment. The formula for FECR as a percentage is as follows: $FECR = 100 - [(post\text{-}treatment\ worm\ egg\ count / pre\text{-}treatment\ worm\ egg\ count) \times 100]$. However, the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) defines resistance as FECR of <95% when reduction analysis was determined 10 days post treatment. DiPietro and Todd (1987) defined resistance as the presence of parasites in fecal samples in more than 20% of horses with less than a 90% reduction. Resistance was defined by Dash et al. (1998) as FECR of less than 80%.

Development of resistance may also be determined by Egg Reappearance Period (ERP) (Sangster, 1999; Tarigo-Martinie et al, 2001). ERP is defined as the time it takes for parasite eggs to reappear in the feces after treatment. Also, determining ERP can be useful in determining the length between dosing intervals (Tarigo-Martinie et al, 2001).

There are several ways to prevent parasite infection and to prevent resistance. A sound deworming protocol should be implemented to the herd. Fecal egg count reduction tests (FECRTs) should be performed yearly. The tests help monitor efficacy of

prevention programs in general and anthelmintic resistance. If the fecal egg count reduction is less than 90%, then there is an indication of anthelmintic resistance. Groups of animals housed together should be dosed at the same time. Failure to dose horses simultaneously leads to ongoing pasture contamination and therefore transmission of infection. Mixing age groups in a pasture will increase the possibility of parasite transmission because the young animals tend to have shorter egg reappearance periods. It is also important to quarantine and deworm all new animals to prevent immediate transmission of parasite infection from external sources.

Resistance to Benzimidazoles

Studies have reported cyathostome resistance to the benzimidazole class over the past several decades (Little et al, 2003). Several studies have reported that resistance is seen after the frequent use of benzimidazoles. For example, Population S, located in Kentucky, found 6 species of cyathostomes resistant to benzimidazoles in a herd administered the benzimidazoles, cambendazole and oxibendazole, every 8 weeks for 4 and 14 years, respectively (Lyons et al., 1996). Adult grazing horses and ponies in Scotland showed evidence of low efficacy of the larvicidal dose of fenbendazole against cyathostomes (mean 14 day FECR 63%) in contrast to high efficacies shown by moxidectin treatment (Chandler and Love, 2002). Reinemeyer et al. (2003) reported horses administered two treatments of the larvicidal dose of fenbendazole within 90 days had significantly greater strongylid fecal egg counts, lower body weights, and poorer body condition scores in comparison to the horses treated with moxidectin.

Resistance to Tetrahydropyrimidines

A large farm in Louisiana showed evidence of cyathostome resistance to pyrantel pamoate with mean FECR of 66.5% (Chapman et al., 1996). On three of 15 farms in Holland, Craven et al. (1998) reported resistance to pyrantel. Furthermore, two of the resistant farms also reported to be resistant to benzimidazoles. In four of 12 farms in north central Florida, pyrantel pamoate had a less than 80% mean reduction in EPG at two and four weeks post treatment, increasing to 9 of 11 and 10 of 11 at 4 weeks (Woods et al., 1998).

Resistance to Avermectin (Ivermectin)

A herd in the Netherlands that had been treated mainly with ivermectin for 10 years has been reported ascarid resistance to ivermectin and moxidectin (Boersema et al, 2002). Hearn and Peregrine (2003) documented ascarid resistance to ivermectin as they found high fecal egg counts in foals after treatment with ivermectin. Hearn and Peregrine (2003) concluded that sole use of ivermectin in ivermectin-resistant ascarid populations increased associated colic and impaction risks in young foals.

Resistance to Milbemycins (Moxidectin)

Not many studies have determined parasite resistance to moxidectin. However, Slocombe et al., (2003) reported the mean efficacy of moxidectin against ascarids was found to be 40.5 % and 53.3% in Thoroughbred and 55.4% in Standardbred foals in Canada.

Rotational Plans

There is a great difference of opinion in the literature on the development of effective rotational programs of anthelmintics. Frequent usage of anthelmintics, using the same class of drug and overstocking all lead to parasite resistance (Bjorn et al., 1992). Several scientists recommend to monitor fecal egg count and only treat when fecal egg count reaches a certain level. However, the scientist disagree on the amount of eggs to determine when the horse should be treated. Suggested levels to treat horses include greater than 100 eggs per gram (EPG) (Uhlinger, 1990; Herd 1993), 200 EPG (Little et al., 2003), or when at least 25% of the horses are over 200 EPG (Uhlinger, 1991).

Slow Rotation

Some slow rotational plans use one class of drug is used for a period of one year followed by the sole use of another the following year. It has been suggested that drugs should be rotated beginning in the winter months when the parasites become more active. Woods (1998) suggested using ivermectin during year one, pyrantel during year two, and a benzimidazole in year three if resistance was not present. Wescott (1986) recommended another slow rotational plan which involved the administration of one class of anthelmintic for a portion of the year, followed by administration of another class of anthelmintic.

Fast Rotation

Fast rotational programs used a different anthelmintic class every time the horse is treated. The horses are treated every 3 to 4 months depending on egg reappearance

period. Intervet, Inc. recommends a rotational deworming schedule to prevent parasites from becoming resistant. During the deep freeze of the winter, the administration of fenbendazole Panacur[®] Powerpack is recommended. This larvicidal dose is 10mg/kg for five consecutive days and is labeled to treat and control all stages of encysted small strongyles. During the past thaw of the spring, it is recommended that ivermectin or moxidectin be administered for general parasite control in addition to bot clearance. Pyrantel pamoate is recommended in the early part of the grazing season for general parasite control. In the late summer horses, this regimen suggests treatment with fenbendazole adulticidal treatment of 5 mg/kg. In the early fall, horses should be administered ivermectin or moxidectin, followed by pyrantel pamoate again in the late fall. Timing is also a key factor of when to dose with the right anthelmintic. The climate and the region will determine the grazing period and will affect the parasite's life cycle. (Intervet Inc., 2000)

Summary

There is a general disagreement in the literature on the extent, cause of, and the control of resistance in equine parasites. Further work is needed to definitively test rotational schemes and strategies to determine efficacy of control of parasitism in the horse.

CHAPTER III

MATERIALS AND METHODS

For the purpose of investigating possible anthelmintic resistance in horses, a herd of Quarter Horses, housed at the Texas Tech Ranch Horse Center in New Deal, Texas, were administered solely fenbendazole every 90 days for 18 months without rotation with any other dewormer. Subsequent to this period, a series of three studies were conducted to determine anthelmintic efficacy and to investigate potential resistance to anthelmintic regimens.

STUDY 1

In September of 2003, 13 Quarter Horse mares, three yearling fillies, eight weanling fillies, and four weanling colts, which had been housed at the Texas Tech University Ranch Horse Center, were randomly assigned to one of three groups. Horses were randomized by fecal egg counts (FEC) which were taken on Day -3, age, and pregnant versus non-pregnant status. All mares were housed in the same pen, had constant access to wheat hay and trace-mineralized salt, and were supplemented with an oats-corn sweet feed grain supplement. The yearlings and weanlings were housed in three different pens according to age and sex. They were all feed alfalfa hay and oat-corn sweet feed and had free access to trace-mineralized salt. All horses had free access to water.

At Day 0, all horses were treated with one of three different doses of fenbendazole (See Figure 2). Group 1 was treated with 5mg/kg BW of fenbendazole

Figure 2. Timeline of Study 1, showing weigh days, fecal sampling, treatment days, serum collection, and body condition score days.

* Horses in Group 3 were treated for 5 consecutive days at each treatment period (Days 0-4, Days 28-32, and Days 56-60)

(Safe-guard[®], Intervet, Inc., Millsboro, DE) on days 0, 28, and 56. Horses in Group 2 were treated with 10 mg/kg BW of fenbendazole (Safe-guard[®], Intervet Inc., Millsboro, DE). This is an off-labeled dose commonly used in the equine industry. Horses in Group 3 were treated with 10 mg/kg BW daily (Panacur Powerpac[®] Paste, Larvicidal Dose, Intervet, Inc., Millsboro, DE) for 5 consecutive days (50 mg/kg BW) during weeks 1, 5, and 9. This dose is recommended for the treatment of encysted larval cyathostomes. Individual doses of anthelmintics were based on individual body weights measured on days -3, 25, and 56 by a certified livestock scale. The required dose for each horse was prepared by adjusting the dial ring of the syringe at the gradation nearest the weight of the horse by rounding up. The paste was administered orally to each horse and care was taken to ensure the full dose was swallowed.

In addition to the baseline sample (day -3), a fecal sample was collected from each horse on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 98, 112, 126 and 140. Quantative egg counts were determined by the Wisconsin Sugar Flotation Method performed by Dr. Gene White, who was blind to the treatments. Results were reported as worm eggs in 1 gram of manure and eggs were identified as ascarids or strongyles.

On day 0, blood was taken by jugular veinipuncture for tapeworm diagnostic testing. Blood samples were allowed to clot for 1 hour and then centrifuged for 10 minutes. One ml of serum was transferred to another labeled tube and sent to East Tennessee Clinical Research, Inc. An ELISA assay was used to measure antibodies against antigens of tapeworms.

A composite fecal sample was taken from the weanlings and yearlings on day 0 to identify and compare resistance to the different anthelmintics. The DrenchRite in vitro

assay was used to detect the larval resistance to benzimidazole, levamisole, benzimidazole/levamisole combination, and avermectin/milbemycin anthelmintics. The nematode eggs were placed into the wells of a microtitre plate, hatched, and the larvae were developed to the L₃ stage in the presence of the anthelmintic. The assay plate contained a single lane for control development (no dye) and eleven lanes which contain increasing concentrations of a drug from each anthelmintic class. The DrenchRite plate was color coded, with green indicating susceptibility, yellow indicating intermediate resistance, and red indicating high resistance. Each drench class was tested in duplicate as follows: benzimidazoles are tested in row A and B, levamisoles in rows C and D, benimidazoles/levamisoles combinations in rows E and F, and the avermectin/milbemycins in rows G and H. The parasite eggs from fecal samples were applied to the wells of the assay plate and hatched. The first stage larvae were fed to sustain development through the infective third stage larvae over the next five days. In the presence of effective drenches, the larval development was blocked. The plate was scanned under a microscope for analysis.

Body Condition Scores (Henneke et al., 1983) for each individual horse were assigned by two trained individuals who were blind to the treatment groups. The scores were averaged and each horse was assigned a score. The scores were assigned on days 0, 28, 56, 84, 112, and 140. Weights were recorded for each individual horse on days -3, 25, 56, 84, 112, 140 by a certified livestock scale.

Statistical Analysis

A randomized block design was used, means were tested by analysis of variance, and separated by least significant difference (Statistix, 2003). FECR analysis was determined 14 days after each treatment by the following equation:

$$\text{FECR} = 100 - [(\text{Post-treatment worm count} / \text{Pre-treatment worm count}) \times 100]$$

STUDY 2

Three months after the last treatment of Study 1 (Day 154), the same horses were re-randomized by fecal egg count taken on Day 140, age, pregnancy status, and previous treatment group and assigned to one of three treatment groups (See Figure 3).

Horses in Group 1 were treated with 0.4 mg/kg BW of moxidectin (Quest[®], Fort Dodge Animal Health, Fort Dodge, IA), Group 2 were treated with 200 mg/kg BW of ivermectin (Zimectrin[®], Merial, Great Valley, NY), and Group 3 was treated with 50 mg/kg bodyweight over 5 consecutive days of fenbendazole (larvicidal treatment). Treatments were given on Day 0 after fecal collections and the dose was determined by the horses' individual weight recorded on day 140.

Fecal samples were collected on Days 154, 161, and 168. Quantative egg counts were determined by the Wisconsin Sugar Flotation Method and was performed by Dr. Gene White who was blind to the treatments. Results were reported as parasite eggs in 1 gram of manure and eggs were identified as ascarids or strongyles.

Statistical Analysis

A randomized block design was used, means were tested by analysis of variance, and separated by least significant difference (Statistix, 2003). Also, chi-square was used to evaluate resistant versus non-resistant status at a 90% reduction level (Statistix, 2003). FECR analysis was determined 14 days after the treatment by the following equation:

$$\text{FECR} = 100 - [(\text{Post-treatment worm count} / \text{Pre-treatment worm count}) \times 100]$$

F = Days fecal samples were collected
T = Treatment Days

Figure 3. Timeline of Study 2, indicating the days fecal samples were collected and day of treatment. Group 1 was administered 0.4 mg/kg BW of moxidectin, Group 2 was administered 200 mg/kg BW of ivermectin, and Group 3 was administered 50 mg/kg BW over 5 consecutive days (Days 154-158).

STUDY 3

Three months after the treatment of Study 2, a year-long practical rotational program was implemented to every horse housed at the Texas Tech Ranch Horse Center which included the stallions, foals, and outside mares (See Figure 4). A quarterly rotation was used by treating all the horses 3 mg/lb BW of pyrantel pamoate (Strongid[®] Paste, Pfizer Animal Health, Exton, PA) in June (n=31), 91 mg/lb BW of ivermectin and 454 mg/lb BW of praziquantel (Zimectrin Gold[®], Merial, Great Valley, NY) in September (n=23), 50 mg/kg BW over 5 consecutive days of fenbendazole (Panacur Powerpac[®] Paste, Larvicidal Dose, Intervet, Inc., Millsboro, DE) in December (n=21), and 0.4 mg/kg BW of moxidectin (Quest[®], Fort Dodge Animal Health, Fort Dodge, IA) in March (n=26).

Treatment doses were determined by weight taken by a trained individual who used certified weight tape. The required dose for each horse was prepared by adjusting the dial ring of the syringe at the gradation nearest the weight of the horse by rounding up. The paste was administered orally to each horse and care was taken to ensure the full dose was swallowed.

Fecal samples were collected on the day of treatment days and seven days after the treatment. Quantitative egg counts were determined as detailed in Study 1 and Study 2.

Statistical Analysis

A randomized block design was used, means were tested by analysis of variance, and separated by least significant difference (Statistix, 2003). Also chi-square was used to determine resistant versus non-resistant status at a 98% reduction level (Statistix,

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W = Weigh day

F = Fecal samples collected

T = Treatment with anthelmintic

June – 3 mg/lb BW of pyrantel pamoate (Strongid[®] Paste, Pfizer Animal Health, Exton, PA)

September - 91 mg/lb BW of ivermectin and 454 mg/lb BW of praziquantel (Zimectrin Gold[®], Merial, Great Valley, NY)

December - 50 mg/kg BW over 5 consecutive days of fenbendazole (Panacur Powerpac[®] Paste, Larvicidal Dose, Intervet, Inc., Millsboro, DE)

March - 0.4 mg/kg BW of moxidectin (Quest[®], Fort Dodge Animal Health, Fort Dodge, IA)

Figure 4. Timeline of study 3. The herd-wide anthelmintic rotational program is illustrated.

2003). Reduction analysis was determined 14 days after the treatment by the following equation:

$$\text{FECR} = 100 - [(\text{Post-treatment worm count} / \text{Pre-treatment worm count}) \times 100]$$

CHAPTER IV

RESULTS

STUDY 1

The DrenchRite assay, based on the initial composite fecal sample, showed mild cyathostome resistance to the benzimidazole class.

Testing for antibodies against antigens of tapeworms with the ELISA assay proved negative.

All ascarids were eliminated by day 7 when analyzed by egg differential analysis, therefore, all study analysis was based on cyathostome egg count.

The body condition scores showed no significant differences between treatment groups at any time measured. (Figure 5)

The analysis of the three treatment groups by fecal egg count reduction 14 days post treatment indicated resistance to the class (Figure 6). On day 14 versus 0, the 5 mg/kg BW group was significantly lower from both the 10 mg/kg BW and the larvicidal group ($P < 0.05$). There was no significant difference between groups on Day 42 versus 28. However, there was a negative reduction in the 5 mg/kg BW and the 10 mg/kg BW group indicating an increase in fecal egg count after the treatment. On Day 70 versus 56, there was a significant difference between the 5 mg/kg and larvicidal groups ($P = 0.10$). Furthermore, there was an increase in fecal egg count 14 days post treatment, thus indicating resistance to this class.

Mean Body Condition Scores						
Treatment	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140
5 mg/kg BW	5.29	5.60	5.78	6.28	6.15	6.27
10 mg/kg BW	5.00	5.45	5.70	6.23	6.08	6.25
50 mg/kg	5.14	5.83	6.08	6.42	6.36	6.32

Figure 5. Mean body condition scores of each treatment group separated by age of young horses (≤ 2 years of age) and mature mares (> 2 years of age).

Figure 6. Fecal Egg Count Reduction in Study 1 in groups of horses administered 5 mg/kg BW fenbendazole (n = 9); 10 mg/kg BW fenbendazole (n = 10); 50 mg/kg BW fenbendazole over 5 days (larvicidal treatment) (n = 9).

All treatments were combined and examined by age of young horses (≤ 2 years of age) and mature mares (> 2 years of age). (Figure 7) Both day 42 versus 28 and day 70 versus 56 showed increase in fecal egg count 14 days post treatment in the young horses after the second and third treatments, indicating resistance in the young horses.

Mean eggs per gram over time (Day 0 - 140) in horses are illustrated in Figure 8. The 5 mg/kg BW and 10 mg/kg BW groups were both significantly different from the 50 mg/kg BW group on Days 7, 70, 77, 84 and 98 ($P < 0.05$). On Days 14, 21, 42, and 126 the 5 mg/kg BW group was significantly different from the 50 mg/kg group ($P < 0.05$). The 10 mg/kg BW group was significantly different from the 50 mg/kg BW group on Day 112 ($P < 0.05$).

Fecal Egg Count Reduction - By Age All Doses of Fenbendazole Treatments

Figure 7. Fecal Egg Count Reduction by age with all treatments combined in Study 1 comparing young horses (≤ 2 years of age) and mature mares (> 2 years of age).

Actual Eggs/Gram by Time Period

Figure 8. Mean eggs per gram by sampling time periods for days 0-140 at every 7 days. Treatment days were 0, 28, and 56.

STUDY 2

Mean FECR percentages tested on day 14 of post treatment was 99.91% for the group treated with 0.4 mg/kg BW of moxidectin, 98.73% for the group treated with 200 mg/kg BW of ivermectin, and 84.30% for the group treated with 50 mg/kg BW over 5 consecutive days fenbendazole (larvicidal dose) (Figure 9). Both the moxidectin and ivermectin groups were significantly different than the group treated with fenbendazole ($P < 0.05$).

The mean fecal egg count reduction of the young horses versus mature mares is illustrated in Figure 10. The young horses treated with moxidectin had a 99.8% reduction and the mares had a 100% reduction. The young horses treated with ivermectin had a reduction of 97.6% and the mares had a 100% reduction. However the young horses treated with fenbendazole had only a 69.9% reduction, whereas the mares had a reduction of 98.7%.

Chi-square analysis was used to evaluate resistant versus non-resistant starts at a 90% reduction level. The ivermectin and the fenbendazole groups were significantly different ($P < 0.05$). The moxidectin and fenbendazole groups were significantly different with $P < 0.01$. However the moxidectin and ivermectin groups were not significantly different from each other.

Fecal Egg Count Reduction of Study 2

Figure 9. Fecal Egg Count Reduction in Study 2 in groups of horses administered moxidectin (n = 11), ivermectin (n = 11), and fenbendazole (n = 11).

Treatment	Age	FECR
Group 1 (MOX)	Young	99.8
	Mature	100
Group 2 (IVM)	Young	97.6
	Mature	100
Group 3 (FBZ)	Young	69.9
	Mature	98.7

Figure 10. Fecal Egg Count Reduction when analyzed by age; young (≤ 2 years) and mares (> 2 years).

STUDY 3

Mean FECR percentages tested on day 7 of post treatment was 95.86% when treated with pyrantel pamoate, 100% when treated with ivermectin + praziquantel, 97.84% when treated with fenbendazole (larvicidal dose), and 100% when treated with moxidectin (Figure 11).

Chi-square was used to evaluate resistant versus non-resistant at both a 90% reduction and a 98% reduction. Mean fecal egg count reductions were not different from both a 90% and 98% reduction at all treatment times.

Treatment	Treatment Month	FECR %
Pyrantel Pamoate (n = 31)	June	95.86
Ivomectin + praziquantel (n = 23)	September	100
Fenbendazole (larvicidal dose) (n = 21)	December	97.84
Moxidectin (n = 26)	March	100

Figure 11. Mean fecal egg count reductions of Study 3 in horses treated with a quarterly rotational program.

CHAPTER V

DISCUSSION

The understanding of cyathostome control is complex because the efficacy of many equine anthelmintic classes can be greatly reduced due to protected encysted mucosal stages of this parasite. Studies over several decades have reported cyathostome resistance to benzimidazoles (Little et al, 2003). The contributing factors to the development of resistance include high frequency of deworming, the continued use of only one class of anthelmintic, and underdosing of the anthelmintic, either by underestimation of the animal's weight or by losing product during the administration.

In Study 1, resistance was documented in the herd after the horses were administered solely fenbendazole for 18 months without rotation prior to the study. This study was in agreement with studies in the literature showing parasite resistance to fenbendazole after prolonged use without rotation (Bjorn et al., 1991; Chapman et al., 1996; Cox et al., 2000; Little et al., 2003; Lyons et al., 1996; Reinemyer et al., 2003; Woods et al., 1998). In the evaluation of fecal egg count reduction at the third treatment period, it was evident that a population of cyathostomes was resistant. It was also evident that the young horses were more resistant to fenbendazole, however, it is important to note that they had an overall higher pre-treatment fecal egg count.

Three months after the last treatment of Study 1, three different anthelmintic regimens were examined to determine efficacy after resistance had been documented in the herd. The treatments of moxidectin and ivermectin both proved to be effective as

their efficacies were all above 98%. However, the horses treated with fenbendazole proved to be ineffective as the reduction of fecal egg counts were below 90%.

As in Study 1 the age effect was very clear. There were no differences between treatment groups (moxidectin, ivermectin, and fenbendazole) in the mature mares. However there was a large difference between the young horses treated with fenbendazole and the mature mares treated with fenbendazole. This indicates a greater problem of cyathostome resistance to fenbendazole in the young horses. This may be due to several factors including decreased immune response in the young horses as well as overall higher egg burdens.

In Study 3, a quarterly rotational scheme was evaluated at controlling parasites in a herd that had been proven to be resistant to fenbendazole. There are great differences of opinions in the literature on the development of effective rotational schemes of anthelmintics with the goal of decreasing resistance. Some researchers suggest that once resistance has been established in an anthelmintic, it is not advisable to go back to that class (Bjorn et al., 1991; Woods et al., 1998). Despite these suggestions, this study showed that resistance in a herd can be broken, parasite resistance can be effectively controlled using a rotation of different anthelmintics, and that a class can be effectively used in this rotational scheme despite prior resistance.

Resistance to every main class of equine anthelmintic has been documented. It is increasingly apparent that parasite resistance to current equine anthelmintics is an area of great concern. It is widely believed that resistance in equine parasites will be an ever increasing problem and a greater understanding is needed for important recommendations to decrease risks of escalating the problem or hastening its speed (Wescott, 1986).

The correct use of anthelmintics and the development rotational programs should be a priority in the equine health world wide. Definitive studies are needed to examine the efficacies of the proposed plans.

CHAPTER VI

CONCLUSION

In summary, many questions still remain regarding parasite control in the horse. There are many varying reports on which type of anthelmintic class should be used to achieve high levels of parasite control and for the prevention of resistance. This study showed that fenbendazole resistance in a herd can be broken with rotation of anthelmintic classes, and that fenbendazole can be effectively implemented in a future rotational program.

However, further research is needed to test and evaluate all aspects of rotational parasite control in the horse. Future studies should be done on different rotational schemes of fast rotation versus slow rotation. This research only involved the fast rotation, whereas, no definitive studies have been done on slow rotational programs.

Future studies will determine how we can slow the problem of parasite resistance and how each anthelmintic class can be used to the fullest efficacy in an equine population.

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