Strongyloïde Infections In Ponies I. Response to Intermittent Thiabendazole Treatments

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ABSTRACT

A group of seven ponies naturally infected with large numbers of small strongyles and raised under conditions to minimize reinfection were treated periodically over a three year span with thiabendazole at the rate of 44 mg/kg body weight. Based on the absence of worm eggs in the feces following each treatment, thiabendazole removed the adult strongyles present with a new population subsequently developing by maturation of inhibited larvae. It took as many as four or five treatments to eliminate or reduce significantly the worm burdens present in the ponies under the conditions of this study.

Strongyle eggs started to reappear in the feces about six weeks after treatment and following the first treatment the mean egg counts rose to the pretreatment level. On successive treatments the interval for worm eggs to appear in the feces lengthened and mean egg counts never rose quite as high as immediate pretreatment levels.

Hematological changes were not marked, although a small steady increase in the mean hemoglobin values and an equivalent small decrease in the mean eosinophil counts occurred in all ponies following each successive treatment.

The study supports the rationale of regular anthelminthic treatment of horses in that even in the absence of reinfection, new burdens of adult worms develop following treatment.

INTRODUCTION

In 1953, Gibson (6) reported on the effect of repeated anthelminthic treatment of six aged horses with phenothiazine. His results showed that there was a tendency for the egg count not to rise as high as it had been before treatment, for fewer worms to be eliminated at each treatment and the interval for the egg count to reach a high level after treatment to lengthen
with each successive treatment. Gibson postulated, particularly in reference to *Trichonema* spp., that the development of large numbers of larvae is inhibited in the histotropic stage and they only leave the mucous membrane of the large intestine after the adult worms in the gut lumen have been removed by the anthelmintic.

With the advent of thiabendazole and indications that it was efficacious against strongyles in horses (4, 10, 17) it seemed an opportune time to determine the effects of repeated usage of this anthelmintic on equines and to study further the phenomenon of inhibited larval development of strongyles. Consequently, a long term study was undertaken in 1966 with the results and observations recorded during the first three years the subject of this paper.

**MATERIALS AND METHODS**

**EXPERIMENTAL ANIMALS**

In the autumn of 1966 seven ponies were purchased for this study. Numbers 1 to 4 were Shetlands, numbers 5 and 6 were Welsh and number 7 was a Welsh X horse cross. All animals were approximately six months of age except 1 and 4, which were one and a half years of age. Four were geldings and three were mares. The ponies originated from three different farms and all were naturally infected with strongyles and *Oxyuris equi*. All ponies except the two older Shetlands were also infected with *Parascaris equorum*.

The ponies were kept tied in individual stalls except for a daily exercise period of one to three hours in an outdoor paddock, depending on the prevailing weather. The stalls were cleaned and swept daily and bedded with either straw or shavings. During exercise periods the ponies were fitted with muzzles to prevent grazing and to minimize reinfection. The ponies were maintained on good quality hay, omolene® and an equine supplement labelled equi-plus®.

**WEIGHINGS**

For the most part, the ponies were weighed at monthly intervals. Following treatment, weighings were carried out at weekly intervals until it was determined if significant changes had occurred or not.

**FECAL EXAMINATIONS**

Fecal examinations were performed twice weekly on fresh feces from all ponies using the McMaster technique or a simple flotation method using supersaturated sodium nitrate as the flotation solution. The simple flotation method was used on all samples in which strongyle eggs were not detected by the McMaster technique.

**Fecal cultures**

Fecal cultures were made of feces from each pony at weekly intervals for the purpose of making differential larval counts. Fecal cultures were not made during periods of negative egg counts following anthelmintic treatments. The culturing procedure involved taking approximately 300 gm of fresh feces, breaking it up as loosely as possible and incubating in a Mason jar with the top loosely in place at 28°C for eight days. On the eighth day the jar was filled with tap water, an inverted petri dish was placed over the opening and the jar was carefully inverted with the petri dish in place. The area of the petri dish surrounding the neck of the jar was filled with tap water and allowed to remain overnight at room temperature. Infective larvae accumulated in the petri dish outside the rim of the jar and were collected with a Pasteur pipette. Random samples were examined and the larvae identified using the keys of Russel (14) and Soulsby (16).

**Hematological examinations**

Hematological determinations included hemoglobin, packed cell volumes, red blood cell counts, eosinophil counts, mean corpuscular volumes and mean corpuscular hemoglobins. Blood samples were taken at monthly intervals and frequently at weekly or biweekly intervals when it was deemed necessary to monitor the ponies more frequently. All samples were drawn into vials.

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1Ralston Purina of Canada Ltd., Woodstock, Ontario.
2Rogar/STB, St. Hyacinthe, Quebec.
containing sufficient ammonium and potassium oxalate crystals to prevent coagulation and change in size of the red corpuscles.

Hemoglobin values were determined colorimetrically using the Klett-Summerson colorimeter and hycel cyanmethemoglobin reagent. Packed cell volumes (PVC) were determined using micropipettes and microhematocrit centrifuge. Red blood cell and eosinophil counts were determined using the appropriate unopette disposable blood diluting pipette and a hemacytometer. The mean corpuscular volume and the mean corpuscular hemoglobin concentration were calculated using values obtained in the above determinations.

ANTHELMINTIC TREATMENTS

Thiabendazole (TBZ) was administered via stomach tube at the rate of 44 mg/kg body weight. The frequency of treatment was dependent upon the pattern of strongyle egg outputs as indicated by the mean fecal counts. Five ponies were treated on five occasions between January 30, 1967 and September 15, 1969 while the remaining ponies (5 and 7) were treated a sixth time on February 10, 1970. The interval between consecutive treatments was 196, 175, 266, 322 and 154 days.

RECOVERY OF PARASITES

Following administration of the anthelminthic, the stall of each pony was carefully cleaned and maintained without bedding. The feces from each animal was collected several times daily into disposable plastic garbage bags over a period of 96 hours. The feces collected from each pony in each 24-hour period was examined for helminths using the method of mixing and sampling outlined by Gibson (5). Samples (ranging from 300 to 450 gm) were mixed with water and passed through a stack of four sieves (mesh sizes 9, 20, 35 and 65). The number of worms in each sample was counted and total count then calculated.

Large strongyles, Parascaris equorum and Oxyuris equi were picked out of the feces and counted during mixing.

RESULTS

CLINICAL OBSERVATIONS

Initially, all ponies were rough coated. Numbers 5, 6 and 7 were thin when purchased. The improvement in the condition of all ponies was marked during the first year. Dull, thick hair coats became sleek and shiny and remained so during the remainder of this study.

WEIGHT GAINS

All ponies showed steady increases in weight which tended to level off in the last year. Numbers 1 and 4, which were one year older than the others at the start of the study, initially weighed more and had a lower rate of gain.

HEMATOLOGY

For the most part, all ponies exhibited a slight increase in the mean hemoglobin values following each anthelminthic treatment. This was particularly marked in the case of pony 7. Conversely, the mean eosinophil counts tended to decrease following each anthelminthic treatment. Mean corpuscular volumes and mean corpuscular hemoglobin concentrations for all ponies were consistently within the range of normal values.

Fecal counts

The mean biweekly strongyle egg counts of the seven ponies are depicted in Fig. 1. Following the first anthelminthic treatment, worm eggs started to appear in the feces about the sixth week. The mean counts rose steadily for approximately four months and levelled off at the mean pretreatment count of about 1500 eggs per gram (epg) of feces. Following the second anthelminthic treatment, the shedding of eggs started.
Fig. 1. The mean biweekly strongyle egg counts of the seven ponies prior to and following each of the anthelmintic treatments with thiabendazole (TBZ).
again in five to six weeks but the mean levelled off at about 200 epg. Following the third anthelmintic treatment, eggs did not start appearing in the feces for about nine weeks and gradually rose to the 200 epg level over the next six months. Following the fourth treatment, eggs did not appear in the feces for about seven to eight months. The mean egg counts did not exceed 50 epg over the next five months. Egg counts following the fifth and sixth anthelmintic treatments were negative.

Fecal Cultures

Infecive larvae of the genera *Trichonema*, *Triodontophorus*, *Gyalocephalus*, *Poteriostomum* and *Delafondia* were recovered. *Trichonema*, *Triodontophorus* and *Poteriostomum* larvae were cultured from feces of all ponies at some time during the study. *Delafondia vulgaris* larvae were cultured from feces of numbers 1, 4, 5, 6 and 7, while *Gyalocephalus capitatus* was only recovered from number 4.

Seventy to 100 percent of the larvae examined from all animals were *Trichonema* spp., except initially in the case of number 7. A very high proportion of the remaining larvae in any sample were *Triodontophorus* spp. Other genera were only recovered in small numbers and in some instances sporadically. *D. vulgaris* larvae were not recovered after the second anthelmintic treatment.

*Trichonema* larvae were consistently the first to appear in the feces following anthelmintic treatment. *Triodontophorus*, *Poteriostomum* and *Gyalocephalus* larvae appeared in the feces from one to two weeks after *Trichonema*. *Trichonema* larvae were the only larvae cultured from feces following the fourth anthelmintic treatment.

Worm Counts

The large (*Delafondia, Alfortia* and *Strongylus*) and small (*Trichonema, Triodontophorus, Poteriostomum, Gyalocephalus* and other strongylid genera) strongyle worms recovered after each treatment are given in Table I. The small strongyles recovered, for the most part, were either *Trichonema* or *Triodontophorus* spp. The maximum number of large strongyles recovered from any pony following treatment
was 25. Most of the large strongyles were *Delafondia vulgaris*, although a few *Alfortia edentatus* and *Strongylus equinus* were present. Large numbers of the pinworm, *Probstmayria vivipara*, were recovered from ponies 4, 5, 6 and 7 after treatment but none from numbers 1, 2 and 3.

The numbers of *Parascaris equorum* and *Oxyurus equi* recovered from any pony were low. *P. equorum* were shed by some ponies even after three TBZ treatments. Ponies 1 and 4 (which were a year older than the others) did not shed ascarid worms. All ponies had *Oxyurus equi* infections with some animals shedding worms even after the fifth treatment.

**DISCUSSION**

Thiabendazole (TBZ) at the recommended dosage rate of 44 mg/kg of body weight was demonstrated to be an effective anthelmintic against adult large and small strongyles, based on the absence of worm eggs in the feces for several weeks following treatment.

The results achieved tend to support Gibson’s hypothesis (6) that it was the removal of adults which stimulated inhibited forms to develop. Dunmore (3), working with *Ostertagia* in sheep, observed a similar phenomenon. However, this almost begs the supposition that the adult population remains static and the evidence for this would appear to be tenuous. In later studies with these same ponies, the author (15) found evidence to suggest that there was a constant turnover in the adult population. Michel (8), working with *Ostertagia ostertagi* in calves, found that the loss of adult worms and their replacement by the further development of fourth stage larvae leads to a frequent exchange of populations of adult worms. Consequently, while there is little doubt that following the removal of adult worms by treatment, a new population develops to replace them, it should not be interpreted to indicate that the presence of adults necessarily prevents arrested larvae from resuming development to the adult stage.

The fact that strongyle eggs started appearing in the feces of the ponies about six weeks after treatment strongly suggests that larvae were indeed present at the time of treatment and were not affected by the drug. Round (12) showed that eggs of small strongyles are present in the feces of treated animals six to seven weeks after dosing but exposure of wormfree ponies to natural infection leads to a patent burden of strongyles at eight to nine weeks. He interpreted the difference of about two weeks between the prepatent period of small strongyles and the period that feces were free from strongyle eggs after TBZ treatment as indicative of a residue of some larvae after treatment.

The failure to recover fourth stage strongyle larvae in the feces following treatment is perhaps not an unexpected finding. Muller (9) found the fourth stage larvae to be associated with nodules in the intestinal wall and several workers have shown that larvicidal activity of thiabendazole against small strongyles is not complete (1, 4, 10, 17). Round (12) postulated that some stages of strongyles present in the intestinal mucosa remain unaffected by the drug.

Treatment with TBZ also removed large numbers of the pinworm, *Probstmayria vivipara*, but this species is regularly shed in small numbers in the feces in the absence of treatment (unpublished data) and this should be taken into consideration. Round (12) also showed TBZ to be very effective against adults of *P. vivipara*. The endogenous nature of the life cycle of *P. vivipara* (7) and the fact that the ponies remained infected throughout this study would suggest that the effect of treatment on the population within the host is perhaps incomplete and of a temporary nature.

The results of this investigation do indicate the value of regular anthelmintic treatment of horses for strongyle infestations. Round (13) showed that in order to control intestinal helminthiasis of horses due to strongyles, treatment must be given at intervals not greater than eight weeks. In this study, under conditions of minimal exposure, it took several treatments over a period of two to three years to eliminate or reduce appreciably the infection present in the ponies. The time required perhaps would have been shorter if this had been the sole objective with treatments administered at shorter intervals.

In the ponies of this study with predominantly small strongyle infections, hematological changes were not marked. A small but steady increase in the mean
hemoglobin values and an equivalent small decrease in the mean eosinophil counts occurred in all ponies following each successive anthelmintic treatment with a further reduction in the worm burdens. On the other hand, Archer and Poynter (2) working with crossbred ponies infected with both large and small strongyles demonstrated a well marked normocytic and normochromic anemia.

Eosinophilia has long been associated with parasitic infections (2, 11, 18). Archer and Poynter (2) reported an eosinophilia of the peripheral blood and bone marrow in ponies which they related to the population of adult strongyle helminths in the lumen of the bowel. In *Ascaris suum* infections in swine the level of blood eosinophils has been shown to be directly correlated to the size of the parasitic challenge (11). In the present study decrease in blood eosinophils would appear to be correlated with a decrease in the worm burden present.

With regard to weight, all ponies showed steady gains from one anthelmintic treatment to the next. While removal of parasites undoubtedly contributed to increases in weight gains, it should be noted that the ponies under study were immature animals and a proportion of the weight gains would be due to normal growth. This was exemplified by the lower rate of gain of the two ponies which were a year older when the study began.

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