Strongyle Infections in Ponies II. Reinfection of Treated Animals

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ABSTRACT

Five of seven ponies whose strongyle worm burdens had previously been removed or markedly reduced by repeated thiabendazole treatments were reinfected with doses ranging from 100,000 to 500,000 small strongyle infective larvae.

Reinfection of ponies resulted in the development of clinical signs characterized by abnormal feces, marked loss of weight and delayed shedding of winter hair coats. An abrupt increase in circulating eosinophils occurred during the first three weeks following reinfection.

Patent infections developed in all ponies with worm eggs appearing in the feces from 12 to 15 weeks after receiving infective larvae. Worm egg outputs followed a cyclic pattern with approximately four to five peaks in egg output per year. There was an abrupt drop in the high worm egg counts in two untreated ponies approximately two and a half years after reinfection. No worms were recovered in the feces of these animals when they were subsequently treated, suggesting that a depletion in the number of inhibited larvae present in these ponies might have occurred.

RÉSUMÉ

Cette expérience visait à réinfester, à l'aide de doses variant de 100,000 à 500,000 larves infestantes de petits strongles, cinq des sept poneys dont on avait auparavant éliminé ou diminué de façon considérable des strongles, à l'aide de traitements répétés au thiabendazole.

La réinfestation de ces poneys provoqua le développement des signes cliniques suivants: fumier anormal, perte de poids considérable et retard de la mue hivernale. Une éosinophilie abrupte se produisit au cours des trois semaines ultérieures à la réinfestation.

Une infestation manifeste se produisit chez tous les poneys dont le fumier recelait des oeufs, à compter de 12 à 15 semaines après l'administration des larves infestantes. L'éliminations des oeufs dans les fèves se produisit de façon cyclique et atteignit de quatre à cinq sommets par année. On nota une baisse soudaine du nombre d'oeufs chez deux poneys non traités, environ deux ans et demi après la réinfestation. On ne recouvra pas de strongles dans les fèces de ces sujets, à la suite d'un traitement ultérieur; cette observation laissa supposer qu'une déplétion du nombre des larves inhibées aurait pu se produire.

INTRODUCTION

In a previous paper (7), the author reported on the periodic anthelmintic treatment of seven ponies which were naturally infected with large numbers of small strongyles. Initially, it was shown that worm eggs started to reappear in the feces about six weeks after treatment and rose to the pretreatment level. On successive treatments the interval of time for worm eggs to appear in the feces lengthened and mean egg counts never rose quite as high as immediate pretreatment levels. Following five or six treatments the ponies shed very few worm eggs in their feces and hematological findings were within the normal range.

In order to determine the host response of sensitized, relatively parasite-free ponies to further strongyle infections five of the seven ponies were reinfected in 1970 with

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Submitted August 21, 1975.

strongyle larvae (predominately small strongyles). This paper deals with this experimental infection.

MATERIALS AND METHODS

Experimental animals

Seven ponies (four Shetlands numbers 1 to 4, two Welsh numbers 5 and 6 and one Welsh x Horse number 7) used in an earlier study on the effects of periodic anthelmintic treatments were again the experimental subjects. Ponies 1 and 4 were five years of age and the remainder were four when the work described in this paper started. They were kept under the same husbandry conditions as previously described (7).

Experimental procedures

Fecal examinations, fecal culturing, hematological examinations, anthelmintic treatments and parasite recovery were carried out using techniques previously outlined (7). Fecal examinations were carried out twice weekly except in a few instances when daily examinations were made to monitor the start of worm egg output. Fecal cultures were done either weekly or bimonthly except in a few instances when they were performed daily. Hematological examinations initially were carried out weekly but later the number of examinations were reduced to one or two per month.

Reinfection of ponies

Infective larvae were cultured from the feces of two horses which were shedding large numbers of strongyle (> 99% *Trichonema* spp. based on examination of infective larvae) eggs in their feces. Infective larvae were washed and placed in 2 litres of tap water. Following thorough stirring, number of larvae/ml was determined (mean of three counts) and appropriate dosages were measured into separate bottles. On March 6, 1970 ponies 1, 3 and 6 were given single doses of 500,000, 100,000 and 300,000 infective larvae respectively via stomach tube. Also starting on March 6, infective larvae were administered to ponies 4 and 5 in divided doses of 20,000 larvae twice weekly until a total of 200,000 was given to pony 5 and 400,000 to pony 4. Ponies 2 and 7 were kept as nonreinfected controls.

Anthelmintic treatment

On November 15, 1971 thiabendazole[1] (TBZ) was administered to ponies 1, 3 and 6 via stomach tube at the rate of 44 mg/kg body weight. On October 1, 1973 TBZ at the rate of 44 mg/kg was given to all seven ponies.

RESULTS

Observations

Following reinfection, pony 1 exhibited anorexia for several days. Starting about a week after reinfection the feces of the two ponies (1 and 6) receiving the largest doses of infective larvae became soft and mushy with loosely formed fecal balls. Loose soft feces was observed from numbers 4 and 5 starting about a month after they were given divided doses of 20,000 infective larvae twice weekly.

During the spring of 1970 the two control ponies (numbers 2 and 7) lost their winter hair coat rapidly. All reinfected ponies retained their winter coats several weeks longer than did the two controls. Furthermore, the new hair coats did not exhibit the sleekness and sheen observed in the nonreinfected ponies. In subsequent years this difference in shedding of winter hair coats was not apparent.

Weight gains

Mean weight gains or losses of the five reinfected and two control ponies during the two yrs immediately following reinfection are given in Fig. 1. During the first eight weeks following reinfection all reinfected ponies lost weight, reaching a mean loss in excess of 50 lbs per animal.

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[1]Equizole, Merck Sharp and Dohme, Montreal, Quebec.

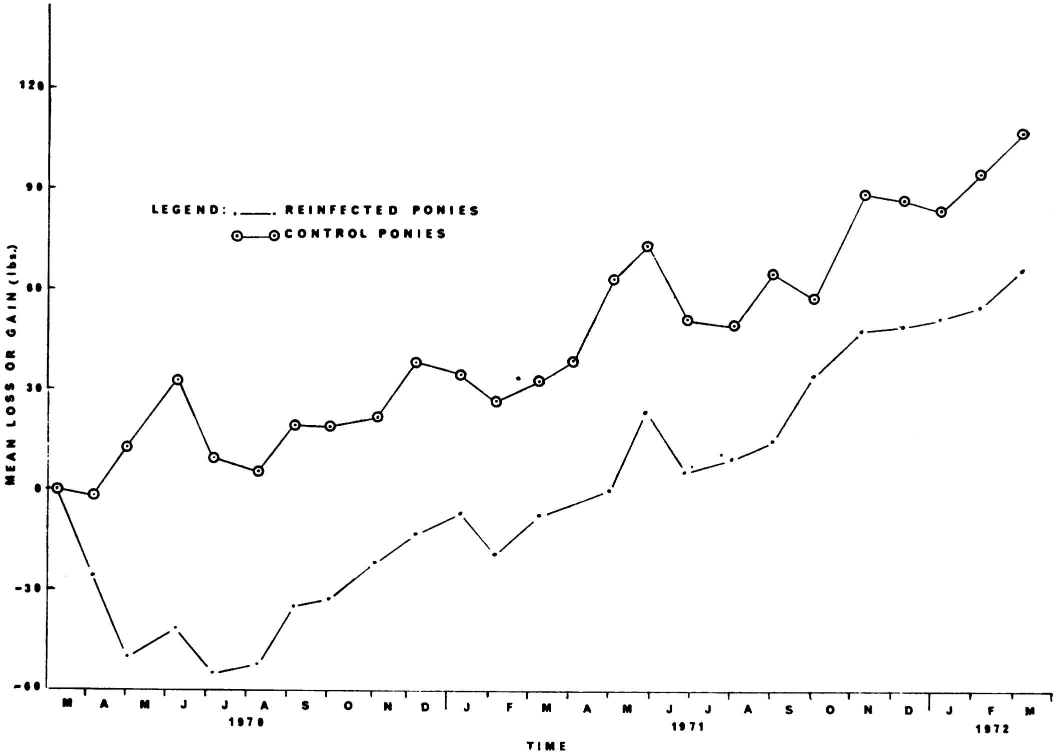

Fig. 1. Mean gain or loss in weight of five reinfected and two control ponies during two years immediately following reinfection.

Without anthelmintic treatment more than a year elapsed before this group of ponies regained the lost weight. On the other hand, the two controls did not exhibit a comparable weight loss.

Hematology

No appreciable changes in mean hemoglobin, erythrocyte and packed cell volume values were observed. However, marked increases in the mean eosinophil counts were observed in all reinfected ponies but not in the controls (Table I). Sharp increases in eosinophil counts were detected in all ponies within a week of reinfection and in most instances reached a peak within three weeks. It is noted that pony 3 which received the smallest dose of infective larvae also exhibited relatively smaller elevations in eosinophil counts.

Fecal counts

The mean biweekly strongyle egg counts per gram of feces (epg) are depicted in Fig. 2. About 12 to 15 weeks after reinfection mean egg counts started to rise in all ponies, reaching a peak at about 30 weeks. High counts did not develop at the same rate in all ponies. High counts first developed in pony 5, followed within a short time in 4 and 6. High counts in pony 1, which had received the highest dose of infective larvae, took at least 12 weeks longer to develop than in any of the other reinfected animals even though a few eggs did appear in the feces about 14 weeks after reinfection. Pony 3, which received the smallest dose of infective larvae had much lower worm egg outputs than the other reinfected animals.

The mean egg counts in the reinfected ponies did not remain static but tended to follow a cyclic pattern reaching a peak about four or five times a year. The cyclic pattern was also observed in the individual egg counts for each pony. The egg output in each cycle was not necessarily of the

Fig. 2. The mean biweekly strongyle egg counts per gram of feces (epg) of reinfected ponies ———, reinfected and treated ponies - - - -, and control ponies . . . . . from 1970 to 1973.

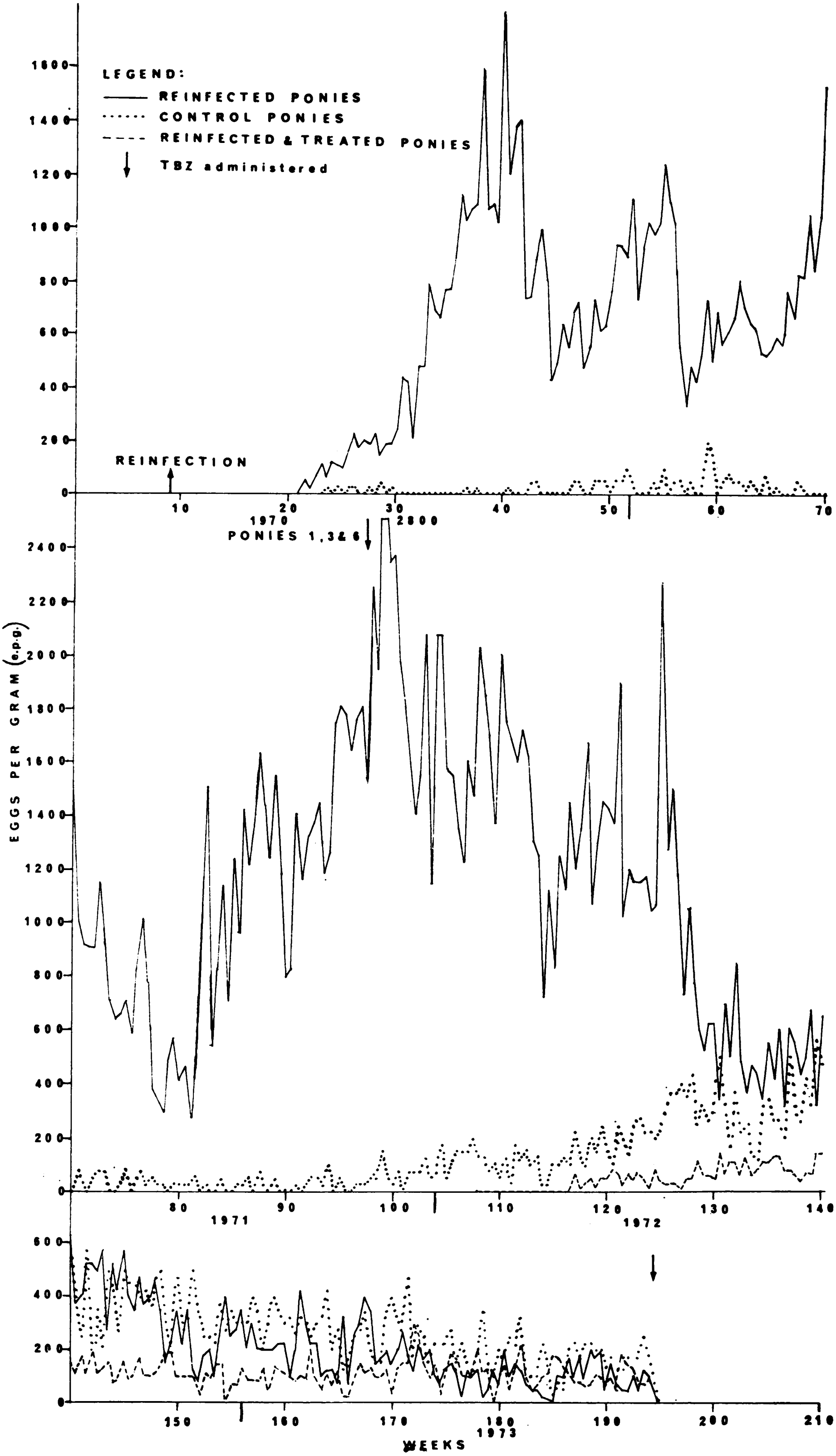
LEGEND:
REINFECTED PONIES
CONTROL PONIES
REINFECTED & TREATED PONIES
TBZ administered
REINFECTION
PONIES 1,3& 6
2800
1970
1971
1972
1973
EGGS PER GRAM (e.p.g.)
WEEKS

TABLE I. The Mean Eosinophil Counts (Eosinophils/cu mm of Blood) for Each Pony During Each Successive Six Month Period Following Reinfection

Pony	Before Reinfection	13/3/70 to 4/9/70	4/9/70 to 5/3/71	5/3/71 to 27/8/71	27/8/71 to 18/2/72	18/2/72 to 8/9/72	8/9/72 to 23/2/73	23/2/73 to 2/9/73
1	304	1851	1897	1941	2395	2157	1213	758
3	123	641	547	509	426	323	234	245
4	175	973	654	541	442	428	374	558
5	197	2027	1481	1171	1026	802	537	696
6	255	1328	1437	1093	1024	944	659	592
Controls								
2	164	142	116	158	134	91	93	91
7	116	62	82	88	60	64	56	51

same magnitude even in the same pony. This is reflected in the mean egg counts (Fig. 2). It should be noted that the initial cycle for ponies 4 and 6 was of considerably greater magnitude than the subsequent two or three cycles. The first three cycles of worm egg output in number 5 were of equal size followed by a number of slightly smaller cycles. In the case of pony 1, the initial cycles in egg counts were smaller than those that occurred later. Towards late summer and fall of 1971 a marked increase in egg counts occurred in ponies 1, 4 and 6. This again is reflected by the mean egg counts (Fig. 2). The egg outputs for pony 3, which received the smallest dose of infective larvae, abruptly dropped to a very low level about 16 months after reinfection. Finally, there was remarkable concurrence in the timing of the egg output cycles among the reinfected ponies (Fig. 2).

Following anthelmintic treatment of ponies 1, 3 and 6 in November, 1971 about 20 months after reinfection, the two remaining nontreated reinfected ponies continued to exhibit a cyclic worm egg output until the summer of 1972 when the mean egg counts abruptly dropped to a low mean value of about 400 to 500 epg. The mean counts continued to decline during the next year.

The mean counts in the two control nonreinfected ponies remained extremely low but a gradual buildup in mean egg counts to about 400-500 epg did occur over a two year period and then dropped to less than 200 epg during the next year.

The three reinfected ponies that were treated in November, 1971 started to shed a few eggs about ten weeks after treatment. The mean egg counts rose to about 100 epg over the next few weeks and continued to fluctuate about this level from then on.

Fecal cultures

Trichonema spp. were the most abundant infective larvae present in all cultures with 95 to 100% of the larvae belonging to this genus. In ponies 1, 2 and 7, *Trichonema* spp. were the only infective larvae recovered. Small numbers of *Oesophagodontus* larvae were recovered from the feces of numbers 3, 4, 5 and 6, *Triodontophorus* and *Strongylus equinus* larvae from numbers 4 and 5, *Delafondia vulgaris* from number 3 and *Alfortia edentatus* from number 5.

Worm counts

Following treatment with TBZ, approximately 20 months after reinfection, almost 7,000 adult worms (mostly *Trichonema* spp.) were recovered from pony 1 and from 6 but none from pony 3. Also large numbers of the pinworm, *Probstmayria vivipara,* were recovered in the feces of numbers 1 and 3 but none from number 6.

At the conclusion of this study in 1973 the pinworm *P. vivipara* was the only parasite recovered from all ponies.

DISCUSSION

This study demonstrates that prior infection with strongyles, particularly small strongyles, does not prevent reinfection or the reestablishment of considerable worm burdens. All reinfected ponies receiving doses of from 100,000 to 500,000 infective larvae developed patent strongyle infections with mean worm egg counts reaching levels comparable to those recorded in the same animals prior to the administration of any anthelmintic treatment (7). Since these ponies were either four or five years of age at the time of reinfection, age does not appear to be a factor. Round (6) similarly had observed that neither age of the pony when infected nor prior infections had apparent effect on the number of eggs passed.

Doses of 100,000 to 500,000 infective small strongyle larvae given either as single or multiple doses were sufficiently large to cause clinical signs of disease in ponies as indicated by the loss of condition and weight, in the delay in shedding and appearance of the hair coat. The unthriftiness as indicated by loss of weight persisted for about five months before the reinfected ponies started to regain weight. It was interesting to note that infections of up to half million small strongyle infective larvae did not have an appreciable effect on the hematological findings in these ponies except that a marked eosinophilia was produced in all reinfected animals.

Roneus (5) working with *Ascaris suum* infections in swine showed the level of blood eosinophils was directly correlated to the size of the parasitic challenge. Archer and Poynter (1) previously reported a peripheral eosinophilia in horses which they related to the population of adult large and small strongyles in the lumen of the bowel. There was an indication in this study that size of the infective dose may also be important in infections with small strongyles in ponies. Pony 3, which received the smallest dose of 100,000 infective larvae, also exhibited the smallest rise in mean eosinophil counts.

In this study a sharp rise in circulating eosinophils was detected within a week of the administration of infective larvae and in all instances had reached peak values by three weeks. Weisman (9) working with *Dictyocaulus viviparus* in calves showed that the time lapse between infection and start of increase in the eosinophil count was five to eight days in the first infection and four to six days in the second. The findings in this study are consistent with those in calves infected with lungworms. On the other hand, Archer and Poynter (1) correlated eosinophilia in horses with egg laying of adult helminths as measured by fecal counts. This was not observed in this study since eosinophilia was present in all ponies from two or three to several months before egg laying started.

There was a variation in time required for the shedding of large numbers of worm eggs even though all ponies were initially reinfected on the same day. Pony 1, which received the largest dose of 500,000 infective larvae took the longest period of time to develop a high worm egg output in the feces. The two control ponies had a low but gradual buildup in worm egg output to about 400 or 500 epg in their feces over a two year period, followed by a drop off in egg counts to about 200 epg during the third year. It is not known if the low rise in egg counts in the two control ponies was due to development of inhibited larvae or due to reinfection in spite of measures taken to minimize such infection. It should be noted that only *Trichonema* larvae were ever cultured from the feces of the two control ponies while a few larvae of other genera were cultured from time to time from the reinfected ponies with the exception of number 1.

The cyclic pattern of the mean worm egg counts with four or five peaks per year would suggest that there is perhaps a turnover in the adult worm population about every ten to 12 weeks. Michel (3) working with *Ostertagia ostertagi* in calves also showed that there was a frequent exchange of populations of adult worms caused by the loss of adult worms and their replacement by the further development of fourth stage larvae. The abrupt cessation of the shedding of large numbers of strongyle eggs in the feces of pony 3 about 16 months after reinfection and ponies 4 and 5 (the two reinfected ponies which did not receive anthelmintic treatment in 1971) about two and a half years after reinfection plus the failure to recover adult worms following subsequent TBZ treatment, would suggest the depletion of the burden of inhibited larvae which presumably were established at the time of reinfection. Another possibility is that some change (perhaps development of resistance) occurred in the host which prevented the establishment and maintenance of a large adult worm population.

There was a remarkable concurrence in the worm egg output cycles among the five reinfected ponies with the possible exception of number 1 which exhibited a marked delay in worm egg production. This was perhaps due to the fact that all ponies were reinfected at the same time although at different dosage levels. However, a point worth noting is that in all instances, patent infections in this study did not develop before 12 weeks after reinfection, although Round (6) showed that a significant egg producing worm burden of small strongyles is present from eight to nine weeks after infection. It must be pointed out that Round (6) used much younger animals that perhaps had little or no previous exposure to strongyle infections. Finally there did not appear to be a detectable difference in the development of strongyle infections in the ponies that could be attributed to either single or repeated administration of infective larvae.

It is interesting to note that all ponies had become infected with *Probstmayria vivipara,* even though this pinworm had not been recorded from ponies 1, 2 and 3 over the three year period of an earlier study (7). The life cycle of *P. vivipara* is completely endogenous with all development taking place in the cecum and colon (8) and large populations apparently are without effect to the host (2). Recent work by the author (unpublished) has shown that infected ponies shed small numbers of *P. vivipara* regularly in their feces. It may be that direct transmission via contaminated water or food occurred despite efforts to minimize this from happening. Because of the endogenous nature of the life cycle of this pinworm, transmission of a very small number of worms perhaps is sufficient to establish an infection.

ACKNOWLEDGMENTS

The technical assistance of Messrs. K. E. Snowdon and D. R. Green is greatly appreciated.

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