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Dose-confirmation studies of the cestocidal activity of pyrantel pamoate paste in horses

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Abstract

Dose confirmation studies of the cestocidal activity of pyrantel pamoate paste were conducted at two sites in North America during 2001. Horses with naturally-acquired cestode infections were identified by detection of typical *Anoplocephala* spp. eggs in feces collected between 7 and 92 days prior to treatment. Twenty and 22 horses were enrolled at Site 1 (Urbana, IL) and Site 2 (Knoxville, TN), respectively. Candidate horses were acclimated to study conditions for 14 days, ranked by length of interval since coprologic confirmation, and allocated randomly to one of two treatment groups: (T1) pyrantel pamoate paste 13.2 mg pyrantel base per kilogram body weight administered orally, and (T2) untreated controls.

Individual doses of pyrantel pamoate paste were prepared on the basis of contemporaneous body weights and administered to Group T1 horses on Day 0. Trained personnel monitored the animals at regular intervals after treatment to detect potential adverse reactions. Horses were euthanatized and necropsied 10–12 days after treatment. The contents of the large and small intestines were collected, and the walls of each organ were rinsed with water and inspected. Attached cestodes were recovered and preserved in 10% formalin. The intestinal contents and rinsed ingesta were washed over a #10-mesh (2 mm aperture) sieve and tapeworms were extracted and preserved. Recovered cestodes were counted and examined at 1–4× magnification for identification to genus and species.

At Site 1, specimens of *Anoplocephala perfoliata* were recovered from seven of 10 control horses, and from one of 10 horses treated with pyrantel pamoate. Mean cestode numbers were 4.52 in the control group and 0.07 for treated horses. At Site 2, cestodes were found in 10 of 11 controls (mean 26.2) and in five of 11 horses (mean 1.2) treated with pyrantel pamoate. In both studies, Group T1 means were significantly lower than the control group ($P < 0.005$). The calculated efficacies were 98.4 and 95.5% at Sites 1 and 2, respectively.

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In two dose-confirmation studies, a single, oral treatment of pyrantel pamoate paste (19.13% w/w pyrantel base) at 13.2 mg/kg was ≥95.5% effective against *A. perfoliata* in naturally-infected horses.

Keywords: Pyrantel pamoate; Anthelmintic; Efficacy; Cestode; *Anoplocephala* spp.; Equine; Horse

1. Introduction

Anoplocephala perfoliata is the most common cestode parasite of equids world-wide (Chapman et al., 2002). The prevalence of infection often approaches or exceeds 50%, as documented in several postmortem surveys (Lyons et al., 1983, 1984; Owen et al., 1988; Benton and Lyons, 1994; Fogarty et al., 1994; Lyon et al., 2000). A seroprevalence survey of over 3300 horses from the United States determined that 54.2% of the animals tested had antibodies (IgG[T]) to *A. perfoliata* (Reinemeyer et al., 2003).

Efforts to control these prevalent parasites began over 30 years ago, when Lyons et al. (1974) investigated the cestocidal activity of tetrahydropyrimidine compounds and reported that the standard, nematocidal dosage (6.6 mg/kg) of pyrantel pamoate was partially effective against *A. perfoliata* infection. At the same dosage, paste and suspension formulations had 88 and 75% efficacy, respectively (Lyons et al., 1989). Slocombe (1979), however, reported that pyrantel pamoate at 6.6 mg/kg only caused destrobilation of *Anoplocephala* and did not remove scolices.

Higher dosages of pyrantel pamoate exhibited superior efficacy against equine cestodes. A dosage of 13.2 mg/kg (i.e., twice the nematocidal dosage) removed 93% (Lyons et al., 1986), 94% (Höglund et al., 1998), 96.6% (Slocombe, 1995), and 97.8% (Slocombe, 1979) of cestodes. Total removal of *Anoplocephala* (Slocombe, 1979) was reported in a single trial with pyrantel pamoate at 19.8 mg/kg (i.e., three times the nematocidal dosage).

Substantial evidence of efficacy notwithstanding, no pyrantel compounds were approved for treatment of equine tapeworm infection in North America prior to the work reported herein. Two pivotal trials were conducted to formally evaluate the cestocidal activity of pyrantel pamoate paste (19.13% w/w pyrantel base) at a dosage of 13.2 mg/kg b.w. against *Anoplocephala* spp. in naturally infected horses.

2. Materials and methods

Two randomized, controlled, anthelmintic efficacy trials were conducted with horses at separate locations during 2001. A common protocol was implemented at both sites. The University of Illinois Veterinary Research Farm in Urbana, IL was designated Site 1, and Site 2 was located at East Tennessee Clinical Research Inc. near Knoxville, TN.

2.1. Animals

Healthy, candidate horses were eligible for enrollment if a fecal sample collected prior to the start of acclimation contained cestode eggs. One horse at Site 1 entered the study 7 days after the acclimation period was initiated.

At Site 1, 20 horses (eight geldings, 11 mares, one intact male) ranging from 1 to 18 years of age were enrolled. Experimental subjects at Site 2 included 11 geldings, eight mares, and three stallions ranging from less than 2 to 20 years of age. Each horse was identified by a uniquely numbered neckband, and by a recorded physical description that included age, sex, breed, and unique markings.

Horses were confined in individual stalls bedded with straw over concrete (Site 1) or hardwood shavings over packed limestone (Site 2). Each stall was equipped with individual grain/hay feeders, and water was available ad libitum.

2.2. Fecal examination

Individual fecal samples were collected from candidate horses on various dates during the 78 days prior to the start of acclimation at Site 1, or during a similar 42-day interval at Site 2. Approximately 40 g of fresh or refrigerated feces were mixed with 30 mL of tap water, and the mixture was strained through two layers of cheesecloth. The filtered liquid was divided

into two portions and each was poured into a 15 mL polyethylene tube and centrifuged at ~1500 rpm for 10 min. The supernatant was decanted and concentrated sucrose (s.g. 1.275) was added until each tube was approximately 2/3 full. The sucrose and sediment were mixed thoroughly with an applicator stick and more sucrose was added until a slight, convex meniscus formed at the top of the tube. A 22 mm × 22 mm coverslip was placed on top of each tube, and centrifugation was repeated at ~750 rpm for 10 min. Coverslips were transferred to microscope slides and examined at 40–100× total magnification for the presence of cestode eggs typical of those produced by *Anoplocephala* spp.

2.3. Allocation

At each site, eligible candidates were ranked in ascending order by length of interval since the most recent, positive fecal examination. Horses with identical intervals were ranked secondarily by ascending identification number, and each two consecutively ranked horses comprised a replicate. Horses within each replicate were assigned randomly to one of two treatment groups: (T1) pyrantel pamoate paste[3] 13.2 mg pyrantel base per kg body weight administered orally, and (T2) untreated controls. Allocation was performed using random numbers assigned by a commercial software program[4].

2.4. Dose preparation and blinding

On Day (−2), a certified livestock scale was used to measure the body weights of all enrolled horses. Individual doses of pyrantel pamoate paste were prepared for animals assigned to Group T1. The investigational product contained 191.3 mg pyrantel base per gram of paste, and individual doses were calculated on the basis of body weight to deliver a target dosage of 13.2 mg/kg, using formula (1):

$$\text{Calculated dose (g)} = \frac{\text{Body weight (kg)} \times 13.2\,\text{mg/kg}}{\text{Concentration}\,[191.3\,\text{mg/g}]} \quad (1)$$

[3] Pyrantel Pamoate Paste, Phoenix Scientific Inc., St. Joseph, MO 64506, Lot Number 0060793.

[4] MicroSoft Office, Excel version 9.0, Redmond, WA.

Doses were prepared by unblinded personnel and rounded to the nearest 0.1 g of paste. Trial personnel responsible for physical examinations, clinical observations, necropsy procedures, and parasitologic techniques were blinded to treatment allocation.

2.5. Treatment

Prior to treatment on Day 0, each horse's mouth was flushed with water to remove any retained feed or hay. The assigned dispensing syringe was introduced into the mouth through the interdental space, and paste formulation was deposited on the surface of the tongue, as far back in the oral cavity as possible. Immediately following treatment, the horse's head was raised for several seconds to encourage swallowing and to minimize rejection. A veterinarian who performed no other functions during the course of the trial administered all doses of paste.

2.6. Health monitoring

Candidate horses were observed once daily on Days −14 through −1 to assess general health, and physical examinations were conducted by a veterinarian 2 days prior to treatment (Day −2). On Day 0, clinical health observations were conducted prior to treatment, and then again approximately 8 h (±2 h) later. Clinical observations were conducted approximately 24 h after treatment, and thereafter once daily until termination of the study.

2.7. Euthanasia and necropsy

On Days 10 and 11 (both sites) and Day 12 (Site 2), complete replicates of horses were euthanatized using methods endorsed by the 2000 AVMA Panel on Euthanasia (Anonymous, 2001). At necropsy, double ligatures were placed at the pylorus, distal ileum, and terminal rectum. The bowel was divided between the ligatures, mesocolic and mesenteric attachments were severed, and the entire small and large intestine were removed from the abdominal cavity and transferred to a labeled container. The large intestine was divided into cecum, ventral colon, dorsal colon, and small colon. Each separate organ was opened lengthwise by sharp dissection and the contents were collected in a large container. The mucosal surface of each incised

organ was rinsed several times with fresh water and washings were combined with the contents. The small intestine was processed similarly, but separately.

The rinsed mucosal surfaces of the small and large intestine were examined, and any adherent cestodes were detached, transferred to labeled containers, and preserved with 10% formalin. The combined large intestinal contents and washings were screened over #10 or smaller ($\leq$2.0 mm aperture) mesh sieves, and any cestodes observed were recovered and preserved. The contents of the small intestine were processed identically.

Recovered cestodes were examined under low-power magnification (1–4× total), counted, and identified to genus and species by trained personnel, using the criteria of Lichtenfels (1975).

2.8. Calculations and statistics

Numbers of *A. perfoliata* recovered from untreated control animals (Group T2) confirmed the sensitivity of the animal selection process, and were used to compute efficacy. The numbers of *A. perfoliata* recovered from treated animals (Group T1) were used to calculate the percentage efficacy and thereby evaluate the objectives of the study.

Total counts of *A. perfoliata* were transformed ($\log_e$[count + 1]) and evaluated statistically via analysis of variance (ANOVA) for a randomized-block design with "Treatment" as a fixed effect and "Block" as a random effect. Group means were compared using ($P < 0.05$) as the level of significance. If a significant difference in mean parasite counts was observed between groups, the percentage efficacy was calculated using formula (2):

$$100 \times \frac{\text{Control}\mu - \text{Treated}\mu}{\text{Control}\mu} = \%\text{Efficacy} \qquad (2)$$

where μ denotes the geometric mean number of *A. perfoliata.*

3. Results

Patent *A. perfoliata* infections were confirmed in 20 horses at Site 1 between 7 and 92 (mean 37.6) days prior to treatment. Patency was documented in 22 horses at Site 2 from 15 to 56 (mean 25.3) days before dosing. Incorporation of a 14-day acclimation period rendered these intervals equivalent to 7 days after to 78 days before the start of acclimation (mean 37.6 days) at Site 1, and 1–42 (mean 11.3) days prior to acclimation at Site 2.

Table 1
Anoplocephala perfoliata counts by treatment group and ranked individual animal

Site	Group	Rank[a]										
		1	2	3	4	5	6	7	8	9	10	11
1	T1	0	0	0	0	1	0	0	0	0	0	n/a
1	T2	3	31	2	0	271	0	13	1	8	0	n/a
2	T1	0	7	14	1	0	1	0	0	0	10	0
2	T2	77	62	32	160	16	72	64	0	5	27	16

[a] Ranked in ascending order by length of interval since coprologic confirmation of infection, and secondarily by ascending identification number.

At Site 1, body weights measured on Day (−2) ranged from 215 to 490 kg (mean 368 kg) and 254 to 510 kg (mean 403 kg) in Groups T1 and T2, respectively. Comparable weights at Site 2 were 230–512 kg (mean 364.9 kg) in Group T1 and 236–493 kg (mean 353.8 kg) in Group T2.

No paste was lost during administration, and all horses in Group T1 received individual doses that delivered 13.2 mg pyrantel base per kilogram body weight.

Specimens of *A. perfoliata* were recovered at Site 1 from seven of 10 control horses and from one of 10 animals treated with pyrantel pamoate (Table 1). At Site 2, tapeworms were found in 10 of 11 untreated controls, and in five of 11 horses from Group T1 (Table 1). Group mean *A. perfoliata* counts and percentage efficacy of pyrantel pamoate are presented in Table 2.

No adverse events were observed at any time after treatment with pyrantel pamoate paste.

Table 2
Mean numbers of *Anoplocephala perfoliata* by group, and percentage efficacy by site

Site	Group[a]	Mean worm counts	Percentage efficacy
1	T1	0.07[b]	98.4%
1	T2	4.52[b]	n/a
2	T1	1.2[c]	95.5%
2	T2	26.2[c]	n/a

[a] Group T1 received pyrantel pamoate paste, 13.2 mg/kg on Day 0; Group T2 was not treated.

[b] Within a column, means with different superscripts were significantly different ($P = 0.0055$).

[c] Within a column, means with different superscripts were significantly different ($P = 0.0003$).

4. Discussion

In two controlled efficacy studies, pyrantel pamoate paste (19.13% w/w pyrantel base) at 13.2 mg/kg b.w. was 95.5–98.4% effective against natural infections of *A. perfoliata* in horses. This range of activity was similar to the results (93–97.8%) of prior studies with the same dosage of pyrantel pamoate (Lyons et al., 1986; Höglund et al., 1998; Slocombe, 1979, 1995).

Because three of 10 control horses at Site 1 and one of 11 at Site 2 were found to harbor no *A. perfoliata* specimens at necropsy, it is apparent that some candidates lost their cestode infections during the interval between fecal diagnosis and postmortem examination. The four uninfected control horses had been confirmed by fecal examination 23, 27, 30, and 92 (mean 43) days prior to Day 0. It is unknown whether the apparent loss of infection was due to parasite attrition or to acquired host immunity. To represent current infection status more accurately, the experimental design of cestocidal studies should incorporate the briefest practical interval between fecal confirmation of tapeworm infection and the beginning of acclimation.

Control horses at Site 1 harbored far fewer specimens of *A. perfoliata* than those at Site 2. This phenomenon remains unexplained because both sites used the same fecal diagnostic procedure to qualify horses, and both studies were conducted contemporaneously during summer, 2001, albeit in different geographic regions. The mean intervals between confirmation and treatment (37.6 and 25.3 days) at Sites 1 and 2, respectively, do not seem sufficiently different to provide a substantial opportunity for natural attrition of cestode populations at Site 1.

It is almost impossible to ensure that equine candidates are adequately infected with *Anoplocephala* spp. because currently available tests cannot determine worm numbers antemortem. Fecal egg counts are poorly correlated to cestode burdens (Proudman and Edwards, 1992; Meana et al., 1998), and an ELISA technique that can correlate serum antibody concentrations to worm numbers (Proudman and Trees, 1996) is not available in the United States. Until definitive diagnostic techniques become available, reducing the interval between qualification and treatment and enrolling more animals than the minimum number necessary are the only practical measures for achieving adequacy of infection.

Because the efficacies of pyrantel pamoate and praziquantel against *A. perfoliata* both exceed 95% (Grubbs et al., 2003; Marley et al., 2004; Rehbein et al., 2004) successful equine cestode control can be achieved with either compound. The availability of alternate classes of cestocides represents an opportunity to avoid or minimize selection for resistance. Anthelmintic susceptibility is not a perpetual characteristic of any parasite population, as confirmed by recent reports of resistance by *Parascaris equorum* to the macrocyclic lactones ivermectin (Hearn and Peregrine, 2003) and moxidectin (Boersema et al., 2002). Anthelmintic resistance has never been reported in *A. perfoliata*, but it would be naïve to assume that this species could not develop resistance to praziquantel or to the pyrantel salts. Among other platyhelminths, resistance to praziquantel has been reported in populations of schistosomes (Brindley, 1994). Furthermore, Kaplan et al. (2004) documented resistance of cyathostomine nematodes to pyrantel pamoate at the nematocidal dosage of 6.6 mg/kg. In the United States, cestocides approved for equine use have been marketed only since 2003, so exposure of *A. perfoliata* populations to praziquantel has not been very intensive to date.

5. Conclusion

These studies demonstrated that pyrantel pamoate paste (19.13% w/w pyrantel base) at a single oral dosage of 13.2 mg/kg b.w. was $\geq$95.5% effective against *A. perfoliata* in horses with naturally-acquired infections.

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