Evaluation of Pyrantel Pamoate, Nitramisole and Avermectin B_1a‡Against Migrating Strongylus vulgaris Larvae

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ABSTRACT

Trials were conducted in ponies to evaluate the efficacy of pyrantel pamoate (Strongid-T®) and two newer anthelmintics not yet commercially available, nitramisole and avermectin B_1a, against migrating Strongylus vulgaris larvae. Ponies were removed from their mares within 24-48 hr after birth and reared in isolation, worm free. Between six and 14 weeks of age they were infected with 2000 or 2500 infective S. vulgaris larvae. Subsequently, they were monitored daily for clinical signs until the experiment terminated at 28 days postinfection. All ponies showed increased body temperature and reduced appetite within the first week of infection. All anthelmintics were administered on day 7 and in addition pyrantel pamoate was given on day 8 postinfection. The anthelmintics were in liquid formulation. Nitramisole and pyrantel pamoate were given by stomach tube and avermectin B_1a by subcutaneous injection.

Following administration of these compounds toxic reactions were not observed. All anthelmintics caused a reduction in body temperature and increased appetite and effected a clinical cure. In ponies which were not treated with an anthelmintic, temperatures remained elevated and appetites never returned completely to normal. These ponies also showed variable degrees of lethargy, depression, recumbency and colic and the majority died between two and three weeks postinfection. At necropsy, these control ponies showed variable degrees of adhesions involving the abdominal organs, necrosis of the ileum and cecum and severe arteritis and thrombosis of the major abdominal arteries and their branches.

Although pyrantel pamoate, used at eight times the therapeutic dose for intestinal nematodes in the horse, effected a clinical cure it did not produce a radical cure. At necropsy, ponies treated with pyrantel pamoate had arteritis and thrombosis of the cranial mesenteric artery and its major branches. Nitramisole and avermectin B_1a were able to effect both a clinical and radical cure.

RÉSUMÉ

Cette expérience visait à déterminer l'efficacité du pamoate de pyrantel (Strongid-T®), ainsi que celle de deux nouveaux anthelminthiques non encore disponibles sur le marché, à savoir le nitramisole et l'avermectine B_1a, à l'endroit des larves de Strongylus vulgaris en migration. On utilisa à cette fin des poneys sevrés dès l'âge de 24 à 48 heures et isolés de façon à les protéger contre les parasites. Lorsqu'ils eurent atteint l'âge de six à 14 semaines, on leur administra 2000 ou 2500 larves infectantes de S. vulgaris. On les surveilla ensuite quotidiennement et jusqu'à la fin de l'expérience qui se termina 28 jours après l'infection, afin de déceler l'apparition de signes cliniques. Tous les poneys expérimentaux manifestèrent de l'hyperthermie et de l'anorexie, au cours de la semaine ultérieure à l'infection. On administra les trois anthelminthiques, sous la forme liquide, le septième jour après l'infection; le lendemain, on répéta le traitement au pamoate de pyrantel. L'administration du nitramisole et du pamoate de pyrantel se fit à l'aide d'un tube oesopha-

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‡Now Ivermectin, formerly Avermectin Dihydro B_1a.

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gien; celle de l'avermectine $B_{1}a$, en injection sous-cutanée.

Aucune réaction toxique ne se développa à la suite de l'administration de ces trois anthelminthiques. Bien au contraire, ils firent disparaître les signes cliniques. Les poneys témoins continuèrent par ailleurs à manifester de l'hyperthermie et de l'anorexie, ainsi que divers degrés de léthargie, de dépression, de décubitus et de coliques; la plupart moururent au bout de deux à trois semaines après leur infection. La nécropsie de ces témoins révéla des adhérences plus ou moins marquées entre les organes abdominaux, de la nécrose de l'iléon et du caecum, ainsi qu'une inflammation thrombotique grave des principales artères abdominales et de leurs ramifications.

L'utilisation du pamoate de pyrantel, à raison de huit fois la dose thérapeutique pour les nématodes de l'intestin du cheval, se traduisit par la disparition des signes cliniques, sans toutefois réaliser une cure radicale. La nécropsie des poneys traités avec cet anthelminthique démontra une inflammation thrombotique de l'artère mésentérique crâniale et de ses principales ramifications. Le nitramisole et l'avermectine $B_{1}a$ permirent par ailleurs d'obtenir une cure tant clinique que radicale.

INTRODUCTION

The life cycle, pathogenesis, pathology, clinical signs, treatment and control of *Strongylus vulgaris* in the equine have been reviewed by McCraw and Slocombe (10) and Ogbourne and Duncan (11) and the detrimental effects of the migrating larval stages are well recognized. Drudge and Lyons (6) found that high dosages of thiabendazole were effective in the acute arteritis syndrome. In this report, an evaluation of three other compounds against migrating larvae of *S. vulgaris* is presented.

Only one of these compounds, pyrantel pamoate (Strongid-T®)[1] is commercially available. This is a broad spectrum anthelmintic effective against several intestinal nematodes and the tapeworm, *Anoplocephala perfoliata*, in the horse (9, 12, 13). Nitramisole is an imidothiazole and an analog of tetramisole with no documented evidence as an effective anthelmintic. The avermectins are a newly discovered group of anthelmintics produced by the actinomycete, *Streptomyces avermitilis* (4). The chemical structures of eight natural components have been described (1). The $B_{1}a$ component has been found to have a wide spectrum of activity against many nematode species (2, 5, 8).

MATERIALS AND METHODS

Pony foals, mainly Shetland-cross, were obtained within 24-28 hrs after birth and reared in isolation. Foals were bottle-fed a milk replacer (Foal-Lac®)[2] until two to three months of age. Solid pelleted feed (15% Horse Feed®)[3] was offered at about two weeks of age and formed the only ration after the milk replacer was withdrawn.

Infective larvae of *S. vulgaris* were obtained by first retrieving adult *S. vulgaris* from the cecum and colon of horses slaughtered at an abattoir. The adults were placed in a 0.5% NaCl solution, identified and cleaned in three changes of 0.5% NaCl. Then either one of two procedures were used. In the first procedure, adult female worms were placed in a solution containing 70% Eagles MEM medium, 20% physiological saline and 10% fetal calf serum in a water bath at 37°C until all the worms had been collected. The worms were then chopped to release eggs and the chopped worms, eggs and medium were mixed with sterile sheep feces and incubated at 26°C for nine to 12 days. In the second method, male and female worms were placed in a dilute electrolyte solution (Pro-ionate®)[4] in a water bath at 37°C until all the worms had been collected. Then the worms were transferred, via a fistula created surgically, into the cecum of a nine to 12 month old pony which was reared and maintained worm free from the previous year. Feces from this pony, containing eggs of *S. vulgaris*, were cultured at 26°C for nine to

[1]Rogar/STB, Division of BTI Products Inc., London, Ontario.

[2]Borden Chemical Inc., Norfolk, Virginia.

[3]Shur-Gain Division, Canada Packers Ltd., Toronto, Ontario.

[4]Burns Veterinary Supply Ltd., Ontario.

12 days. Infective larvae were harvested from the cultures in both procedures and stored in 0.5% saline at 6°C. When required the larvae were counted and inoculated into ponies via a silicone-coated stomach tube inserted through the nostril. The tube was rinsed thoroughly with water before removal from a pony.

After ponies were infected they were monitored daily for temperature, appetite and abnormal signs. The anthelmintics were administered at the end of the first week postinfection (PI). After treatment, ponies were observed for the first hour and periodically for the next 24 hr for toxic drug reactions. A necropsy was performed on day 28 PI or shortly after a pony had died.

Trials with nitramisole[5]

Four ponies were used in this trial and were randomly allocated to two groups. All ponies were infected, each with 2000 infective larvae. Nitramisole was administered on day 7 to one group via a stomach tube at the rate of 8 mg/kg.

Trials with pyrantel pamoate

Two ponies were infected, each with 2500 infective larvae. On day 7 and 8 PI, pyrantel pamoate was administered to one pony using a stomach tube at the rate of 52.8 mg pyrantel base/kg.

Trials with avermectin B_1a[6]

Eight ponies were used in this trial and these were allocated, on the basis of body weights, to two groups. One group contained the four heavier and the other the four lighter ponies. Within each group, ponies were randomly allocated to one of four treatment subgroups. All ponies were infected, each with 2000 infective larvae. Treatments involved one of three doses of avermectin B_1a or a placebo. Both materials were in liquid formulation for subcutaneous injection. Avermectin B_1a was administered at the rate of either 100, 300 or 800 μg/kg. The placebo was regarded as containing a substance of equivalent concentration and was administered at the rate of 300 μg/kg. All treatments were given on day 7 PI. Avermectin B_1a, except at 800 μg/kg, and the placebo were given in one site subcutaneously in the midlateral neck region. The total volume of avermectin B_1a at 800 μg/kg was large and it was divided and equal amounts were given on either side of the neck. Body weights of all ponies were measured at the commencement and end of the trial and at the time of treatment. At necropsy, the cranial mesenteric, ileocolic and the first 2.5 cm of the major branches of these arteries were searched using a dissecting microscope for larvae which were recovered and counted. If thrombi were present they were removed and examined for larvae. After searching for and collecting larvae, the dissected arteries and thrombi from two ponies (44/78 and 54/78) were placed in pepsin HCl at 37°C to determine whether additional larvae could be recovered. The digest was examined five and 17 hr later.

RESULTS

All ponies showed an increase in body temperature during the first week PI, some as early as day 2 PI. In the five untreated ponies this increased body temperature continued throughout the remainder of the experimental period with three ponies dying before day 28 PI. These ponies also exhibited lethargy, depression and recumbency and in one pony (20/76) periodic attacks of colic.

All ponies showed a reduction in appetite during the first week PI, some as early as day 2 PI. In four of the five ponies not treated with an anthelmintic the appetite continued to decrease as the experiment progressed and eventually these ponies became anorexic.

At necropsy, the untreated ponies showed extensive and tenacious adhesions involving variable amounts of small and/or large intestine and body walls. In the three ponies which succumbed before day 28 PI there was in addition severe gangrene of the ileum and in pony 20/76 also of the cecum. In all these ponies, there was extensive arteritis and thrombosis of the cranial

[5]Supplied by Pitman-Moore Inc., Washington Crossing, New Jersey.

[6]Supplied by Merck Sharp and Dohme, Rahway, New Jersey.

mesenteric artery and its major branches and sometimes the celiac artery (Fig. 1). The lesions found in these untreated ponies as well as in ponies receiving anthelmintics are summarized in Table I.

TRIALS WITH NITRAMISOLE

In the treated ponies, body temperatures returned to normal within 24-72 hr after treatment (day 8-10 PI) and full appetite

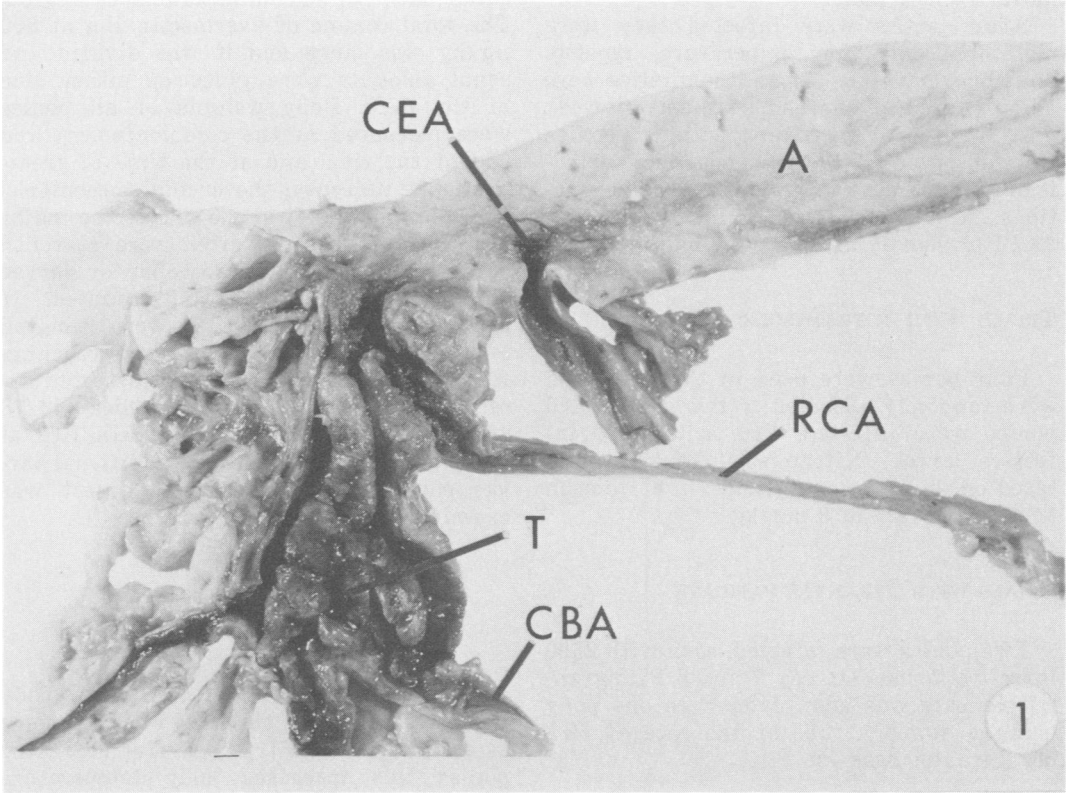

Fig. 1. Arteritis and thrombosis of the cranial mesenteric artery and its major branches and of the celiac artery of pony 48/78 which was given a placebo. A-aorta, CBA-colic branch of the ileocolic artery, CEA-celiac artery, RCA-right colic artery, T-thrombus.

TABLE I. Some Data on Experimental Infections, Chemotherapy and Necropsy Findings

Treatment	Dosage /kg	Pony No.	Age at Infection Days	Necropsy		
				Days Postinfection	Lesions	
					Viscera	Arteries
Nitramisole	8 mg	16/76	100	28	None	None
	8 mg	31/76	50	28	None	Slight
None		19/76	100	34	Extensive	Severe
		20/76	89	16 (Died)	Severe	Severe
Pyrantel base	52.8 mg	45/77	42	28	Slight	Severe
None		42/77	44	21 (Died)	Extensive	Severe
Avermectin B_1a	100 μg	47/78	84	28	None	None
	300 μg	46/78	82	28	None	None
	800 μg	50/78	64	28	None	None
Placebo		48/78	89	28	Slight	Severe
Avermectin B_1a	100 μg	44/78	84	28	None	None
	300 μg	53/78	52	28	None	None
	800 μg	45/78	83	28	None	None
Placebo		54/78	41	21 (Died)	Extensive	Severe

was regained by day 12-13 PI. Toxic reactions were not observed. At necropsy, the viscera and the cranial mesenteric artery and its major branches were normal in pony 16/76 and only the ileocolic artery was affected in pony 31/76. This was slightly dilated with a corrugated intimal surface and there were two small fibrin tracks on the intima of the aorta near the opening of the cranial mesenteric artery.

Trials with pyrantel pamoate

In the treated pony, body temperatures returned to normal by 96 hr (day 12 PI) after the second treatment and full appetite resumed after treatment (day 8 PI). Toxic reactions were not observed. At necropsy, there were enlarged hemorrhagic lymph nodes associated with the cecum and colon, an adhesion anchoring the apex of the cecum to the ventral mid-line, arteritis and thrombosis of the cranial mesenteric artery and its major branches and fibrin tracks of migrating larvae on the intima of the aorta (Fig. 2).

Trials with avermectin B_{1a}

In ponies treated with avermectin B_{1a} at 300 and 800 $\mu g/kg$, temperatures declined to normal within 24-72 hr (day 8-10 PI) after treatment. Temperatures declined more gradually in the ponies treated at the rate of 100 $\mu g/kg$. All these ponies regained full appetite by day 10 or 11 PI. The data for weights of ponies are shown in Table II. All ponies, except pony 54/78, showed a reduction in weight by day 7 PI. Ponies treated with avermectin B_{1a} had a terminal weight greater than that for day 0. Pony 48/78 which received a placebo had a terminal weight lower than that at day 0 and the other pony, 54/78, died on day 20 PI and a terminal weight was not taken.

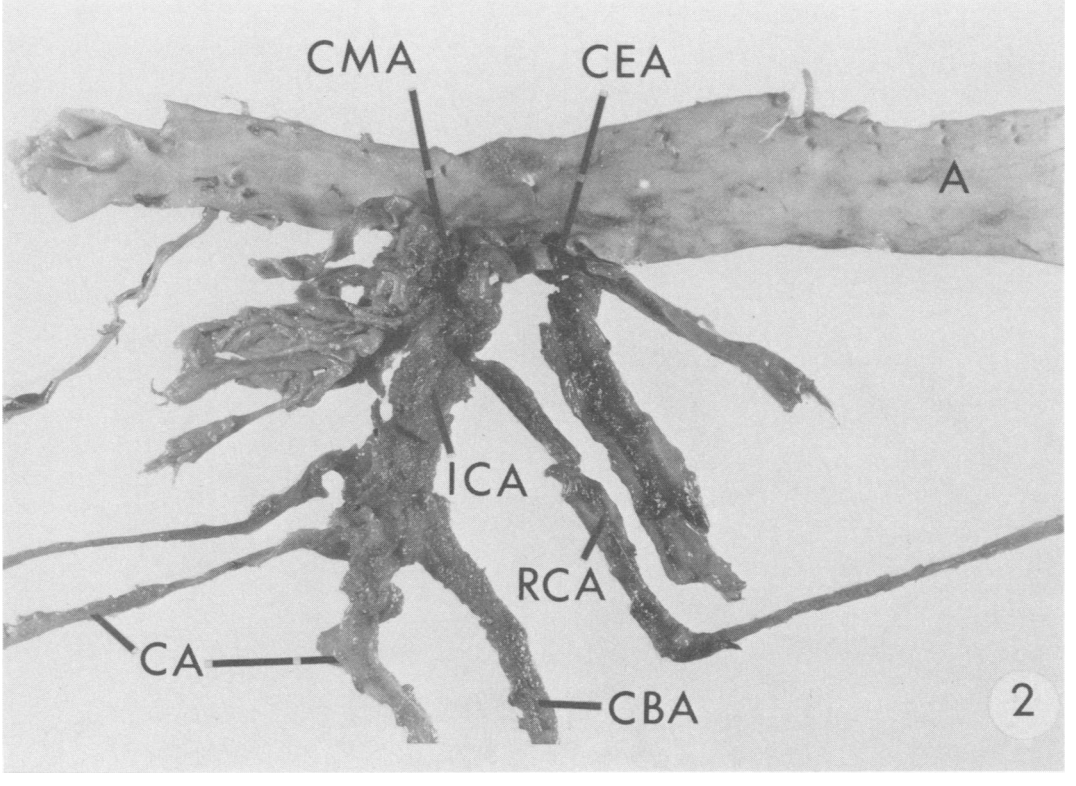

Fig. 2. Abnormal arteries of pony 45/77 treated with pyrantel pamoate. Fibrin tracks were present on the intima of the aorta (A). Arteries which were dilated and corrugated included: CMA-cranial mesenteric artery, RCA-right colic artery, ICA-ileocolic artery, CBA-colic branch of the ileocolic artery, CA-cecal arteries, CEA-celiac artery and its branches.

Ponies 45/78 and 50/78, which had received the highest dose rate (800 μg/kg), showed some reaction at the site of injection. A soft swelling about 3 cm thick occurred on the right side of the neck within one hour of administration. The size of the swelling decreased dramatically within 24 hr and retracted to a small lump by 48 hr. In pony 50/78 this could still be detected on day 28 PI. At necropsy, there was a slight thickening of the dermis but other tissues appeared normal.

TABLE II. Weight (kg) of Ponies on Three Occasions in Trials with Avermectin B_1a

Pony ID	Avermectin B_1a μg/kg	Comm. of Study	Days Postinfection	
			7	28
47/78	100	42.0	37.5	43.0
46/78	300	38.5	38.4	42.5
50/78	800	39.5	38.0	43.0
48/78	Placebo	37.5	36.0	33.0
44/78	100	23.0	21.5	26.0
53/78	300	37.0	36.5	40.0
45/78	800	34.0	31.5	36.5
54/78	Placebo	32.0	33.0	—

At necropsy, no other lesions were found in any of the ponies treated with avermectin B_1a (Fig. 3) and no larvae were recovered from the cranial mesenteric artery or its major branches. In pony 54/78 which received a placebo, 141 fourth stage larvae were recovered from the cranial mesenteric artery and its major branches. Nine additional larvae were recovered after the dissected arterial scrapings and thrombi were placed in 100 mL of pepsin HCl for five hours and a further 14 larvae after a further 12 hr. In pony 48/78 which also received a placebo, a total of 279 larvae were recovered from the arteries.

DISCUSSION

Ponies infected with *S. vulgaris* showed increased body temperature and reduced appetite within the first week of infection

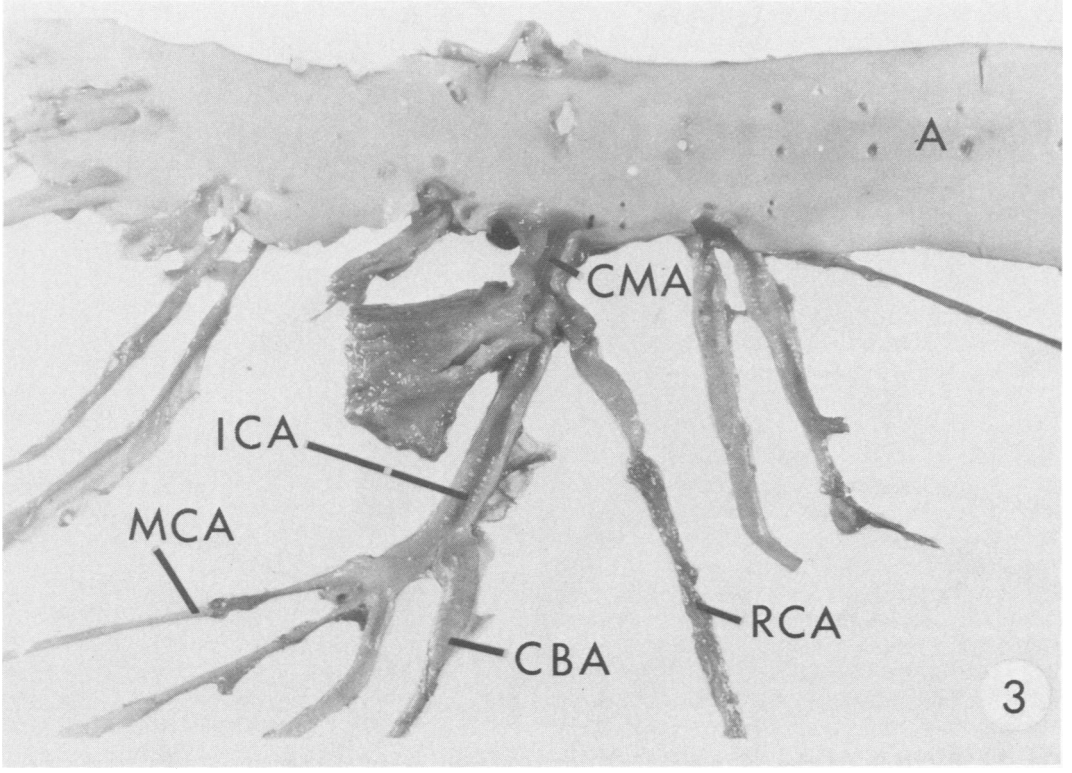

Fig. 3. Normal arteries of pony 47/78 treated with avermectin B_1a. A-aorta, CBA-colic branch of the ileocolic artery, CMA-cranial mesenteric artery, ICA-ileocolic artery, MCA-medial cecal artery, RCA-right colic artery.

which was similar to the findings reported by Drudge *et al* (7) who used high doses of infective larvae. Drudge and Lyons (6) treated such infected ponies on day 7 and 8 PI and in untreated ponies death occurred as early as day 14 PI. Pyrantel pamoate, nitramisole and avermectin $B_{1}a$ when administered at the end of the first week PI prevented the development of the acute arteritis syndrome associated with the migrating larvae. These anthelmintics were, therefore, able to effect a clinical cure.

In the five untreated ponies, body temperature remained elevated and the appetite never returned to normal. There were variable degrees of lethargy, depression, recumbency and colic and three ponies died between two to three weeks PI. At necropsy the ponies showed extensive adhesions involving various parts of the intestine and the body wall as well as severe arteritis and thrombosis of the cranial mesenteric artery and its branches. In the three ponies which died there was, in addition, necrosis of the ileum.

Toxic reactions were not observed when pyrantel pamoate was used at eight times the therapeutic dose for intestinal nematodes in the horse. Dose rates of up to 20 times the therapeutic dose have produced no adverse effects (14). Although high doses of pyrantel pamoate were able to effect a clinical cure, it did not produce a radical cure. At necropsy, ponies were found with severe arteritis and thrombosis of the cranial mesenteric artery and its major branches. Drudge and Lyons (6) found that the water soluble and more highly absorbable hydrochloride salt of pyrantel at 40 mg pyrantel base/kg was able to effect a radical cure but it produced toxic signs.

Toxic signs were not observed with nitramisole which was able to effect a near radical cure. In one pony, there was minimal arteritis of the ileocolic artery and two fibrin tracts were found in the aorta. Toxic signs were not evident following any of the three dose rates of avermectin $B_{1}a$. This compound promoted both clinical and radical cures and the small dosage needed to effect these was remarkable. As an injectable anthelmintic for horses, it would be an extremely useful product. There are at present, therefore, compounds which have considerable promise in the treatment of *S. vulgaris,* at least in the early stages of infection.

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