Anthelmintic Activity of Tetramisole, Thiabendazole, and Purified Fine Particle Phenothiazine Against Experimental Infections of *Haemonchus contortus* and *Trichostrongylus* Species in Sheep

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The efficacy of tetramisole (2,3,5,6-tetrahydro-6-phenyl-imidazo [2,1-b] thiazole hydrochloride), a broad spectrum anthelmintic for man and animals, was first reported by Thienpont et al. (1966). Rohrbacher et al. (1967) and Bullock et al. (1968) reported that the original optically inactive dl-tetramisole had been separated into optically active d and l compounds and that anthelmintic activity was ascribable to l-tetramisole. In preliminary trials, l-tetramisole had about the same anthelmintic activity as the dl compound when given at about half the dose rate. Published data on the activity of tetramisole in sheep and goats pertain to dl-tetramisole; see Cornwell et al. (1967), Fitzsimmons (1966), Forsyth (1966), Gibson (1966), Guilhon (1966), Lyons et al. (1968), Pankhurst and Sutton (1966), Reinecke (1966), Ross (1966), Shone and Philip (1967), Thomas and Bainbridge (1967), and Walley (1966). Because of the marked promise shown by tetramisole as an anthelmintic for sheep, a trial was conducted to compare the activity of the dl and l compounds with that of thiabendazole and phenothiazine against experimental infections of *Haemonchus contortus*, *Trichostrongylus axei*, and *Trichostrongylus colubriformis* in sheep.

**Materials and Methods**

**Experimental animals:** Polled Dorset lambs (18 wethers and 2 females) used in this trial were 10–10.5 months old, weighed 21–32 kg (avg 27.7 kg), and were raised parasite-free except for insignificant infections of *Strongyloides papillosus* and coccidia. The lambs were allocated to five groups of four lambs, each group having comparable mean weights (Table 1). Each group was held in separate, concrete-floored pens which were thoroughly cleaned every 2 days. The lambs were fed a balanced, pelleted ration in adequate quantity to maintain moderate but not maximum growth.

**Origin and enumeration of infective larvae:** An isolate of *H. contortus*, designated AH-2, was established in 1966 from the sheep flock at the Animal Husbandry Division, Agricultural Research Service, Beltsville, Maryland. Infective larvae used in this trial were obtained from standard cultures of feces from a stock lamb infected with this isolate.

Infective larvae of *Trichostrongylus* spp. were of the KH isolate (Kates and Thompson, 1967), which contained, on the basis of previous sheep necropsies, about 28 per cent *T. axei* and 72 per cent *T. colubriformis*.

Prior to larval inoculation of the lambs, infective larvae in the two suspensions (one of *H. contortus* and one of *Trichostrongylus* spp.) were quantitated as follows: After appropriate dilution, the larvae in ten 1-ml samples from each suspension were counted in Scott counting chambers; undiluted formalin was added to immobilize the larvae. The per cent standard error (se) of the larval counts of the *H. contortus* suspension was 1.87 and of the *Trichostrongylus* susp. was 1.55.

**Larval dose preparation and inoculation of lambs:** Each larval dose per lamb contained 5,000 ± 93 se *H. contortus* and 44,400 ± 688 se *Trichostrongylus* spp. (95% confidence interval = se × 2.26). Each dose was concentrated by centrifugation and pipetted onto filter paper. The latter was then inserted into a gelatin capsule and administered by balling gun.

**Anthelmintics employed, their administration, and dosages:** (1) Tetramisole: chemically pure l and dl compounds; American Cyanamid Co., Princeton, N. J. (2) Thiabendazole: Thibenzone; Merck & Co., Rahway,
Table 1. Activity of anthelmintics against *H. contortus* based upon necropsy worm counts.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of lambs(^1) (Avg wt, kg)</th>
<th>Dose rate mg/kg</th>
<th>Adult Avg (range)</th>
<th>4th-stage larvae Avg (range)</th>
<th>Total worms Avg (range)</th>
<th>Per cent efficacy 5th-stage</th>
<th>4th-stage$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Tetramisole</td>
<td>4 (28.8)</td>
<td>8</td>
<td>0</td>
<td>(0–20)</td>
<td>(0–20)</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>dl-Tetramisole</td>
<td>4 (26.8)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>4 (27.5)</td>
<td>50</td>
<td>77</td>
<td>1,470</td>
<td>1,547</td>
<td>65</td>
<td>3</td>
</tr>
<tr>
<td>Phenothiazine</td>
<td>4 (27.6)</td>
<td>550</td>
<td>&lt;1</td>
<td>(0–3)</td>
<td>(160–3,120)</td>
<td>99</td>
<td>8</td>
</tr>
<tr>
<td>purified, 2–3 $\mu$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4 (27.7)</td>
<td></td>
<td>226</td>
<td>1,520</td>
<td>1,746</td>
<td>(143–393)</td>
<td>(920–2,040)</td>
</tr>
</tbody>
</table>

\(^1\) All males (wethers), except one female each in dl-tetramisole and thiabendazole groups.

\(^2\) See text.

N. J. (3) Phenothiazine: 2–3 $\mu$ average particle diameter; purified grade (approximate purity 99.9%); wetting agent 1% lecithin; Atomic Basic Chemical Co., Pittsburgh, Pa.

All the anthelmintics were prepared as aqueous drenches and administered with a dose syringe on the 28th day postinfection. The two commercial products were given at commonly used dose levels, thiabendazole at 50 mg/kg and phenothiazine at 550 mg/kg. *dl*-Tetramisole and *l*-tetramisole were given at 15 and 8 mg/kg, respectively.

**Experimental Procedures:** Data on weight gains, packed red-cell volume (micro-hematocrit), and worm eggs per gram of feces were obtained weekly on all lambs.

All lambs were killed 1 week after treatment. The parasites remaining in the treated and control lambs were recovered and counted by the following procedures: The contents of each abomasum and small intestine were recovered and stored in a large container to which undiluted formalin was added. Each organ was then separately subjected to artificial peptic digestion overnight at 37 C. The next day the contents and digests of each organ from each lamb were combined, and the final preparations made up to 2,000 ml for sampling. From each preparation, five 20-ml samples were taken for calculating the numbers of *Trichostrongylus* spp. and larval parasites in each organ. After the samples were removed from the total abomasal material, the remainder was washed through screens and the adult *H. contortus* recovered. All adult *H. contortus* were counted, but total numbers of the small nematodes were calculated from the sample counts.

Efficacy calculations were made by the standard method for the controlled anthelmintic test.

**Supplemental Lamb Infections:** Because substantial numbers of apparently inhibited 4th-stage *H. contortus* were recovered 35 days postinfection from lambs in the control and two treatment groups (Table 1), four more lambs were employed to determine the inhibitory influence, if any, of simultaneous infection of *Trichostrongylus* spp. on the development of *H. contortus*. Two lambs were inoculated with similar numbers of larvae of *H. contortus* and *Trichostrongylus* spp. (5,000 and 44,200, respectively) of the same isolates that were used in the anthelmintic trial, and each of two lambs was inoculated with 5,000 *H. contortus* larvae only (Table 4). Subsequently, these lambs were penned, fed, necropsied, and their parasites recovered and counted in the same manner as for the lambs in the anthelmintic trial.

**Results**

This trial was planned to obtain comparative data on the activity of certain anthelmintics against adult populations of three important nematode parasites of sheep. Consequently, the lambs were treated 28 days postinfection when practically all worms ordinarily should have become adults. Surprisingly, however, the majority of the *H. contortus* recovered at necropsy from the control lambs and from two of the four treatment groups (thiabendazole and phenothiazine) were 4th-stage larvae (Table 1). Therefore, for this and other reasons discussed later, we believe that considerable numbers of inhibited 4th-
stage *H. contortus* were present in lambs of all 5 groups when the anthelmintics were administered. If this assumption is correct, data were obtained on the activity of the drugs against 4th-stage *H. contortus* as well as adults.

The data on the activity of the anthelmintics against adult and 4th-stage *H. contortus* are summarized in Table 1. Both dl- and l-tetramisole were 100% effective against adult *H. contortus* and almost as effective against 4th-stage larvae; only one 4th-stage larva was found in 20 abomasal samples from the four lambs given l-tetramisole. Phenothiazine was 99% effective against adult *H. contortus*, but was ineffective against 4th-stage larvae. Thiaabendazole was only 65% effective against adult *H. contortus* and almost totally ineffective against 4th-stage larvae.

Data on activity of the anthelmintics against *T. axei* and *T. colubriformis* are summarized in Table 2. All were very effective (94–100%) against adults of both species.

All anthelmintics reduced the parasite egg counts 92–100% (Table 3). Thiabendazole caused the least reduction because of the retention of adult *H. contortus* after treatment. No eggs were seen in the posttreatment fecal samples from lambs treated with tetramisole. The drugs were well tolerated in all trials. Average pretreatment egg counts in the five groups of lambs were quite uniform, varying only from 2,445 EPG in the group given phenothiazine to 3,563 EPG in the control group. This uniformity in the pretreatment egg counts indicated that reasonably uniform adult infections were established initially in the lambs. However, an average of 226 adult *H. contortus* (55% females) were present in the control lambs at necropsy, whereas the same lambs had an average of 14,470 adult *Trichostrongylus spp.* (54% females) (Tables 1, 2). Therefore, substantial numbers of the eggs counted pretreatment were probably those of *Trichostrongylus spp.* No attempt was made to differentiate the eggs of *H. contortus* and *Trichostrongylus spp.* because quantitative differentiation of *T. axei* and *H. contortus* eggs in mixed infections is unreliable.

The reduction in average hematocrit values over the 4 weeks from infection to treatment

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### Table 2. Activity of anthelmintics against *Trichostrongylus* spp. based upon necropsy worm counts.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of lambs</th>
<th>Dose rate mg/kg</th>
<th><em>T. axei</em> Avg (range)</th>
<th><em>T. colubriformis</em> Avg (range)</th>
<th>Both species Avg (range)</th>
<th>Per cent efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Tetramisole</td>
<td>4</td>
<td>8</td>
<td>65</td>
<td>0</td>
<td>65</td>
<td>98</td>
</tr>
<tr>
<td>dl-Tetramisole</td>
<td>4</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Thiaabendazole</td>
<td>4</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Phenothiazine purified, 2–3 μ</td>
<td>4</td>
<td>550</td>
<td>(0–60)</td>
<td>(140–1,260)</td>
<td>(200–1,300)</td>
<td>99</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>–</td>
<td>3,875</td>
<td>10,975</td>
<td>14,470</td>
<td>94</td>
</tr>
</tbody>
</table>

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### Table 3. Activity of anthelmintics against *H. contortus* and *Trichostrongylus* spp. based upon combined species fecal egg counts.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of lambs</th>
<th>Dose rate mg/kg</th>
<th>Avg EPG</th>
<th>EPG reduction per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Tetramisole</td>
<td>4</td>
<td>8</td>
<td>3,550</td>
<td>0</td>
</tr>
<tr>
<td>dl-Tetramisole</td>
<td>4</td>
<td>15</td>
<td>3,508</td>
<td>0</td>
</tr>
<tr>
<td>Thiaabendazole</td>
<td>4</td>
<td>50</td>
<td>3,825</td>
<td>205</td>
</tr>
<tr>
<td>Phenothiazine purified, 2–3 μ</td>
<td>4</td>
<td>550</td>
<td>2,445</td>
<td>30</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>–</td>
<td>3,563</td>
<td>2,645</td>
</tr>
</tbody>
</table>

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1 Same lambs inoculated simultaneously with *H. contortus* (Table 1).
2 All *Trichostrongylus* spp. recovered were adults.
port s out conclusion that average infections per group at the time of treatment were varied only from 5–6% per group, which supports our conclusion that average infections per group at the time of treatment were comparable.

Our conclusion that the 4th-stage *H. contortus* larvae recovered at necropsy were derived from the original inocula is supported by the fact that similar percentages of *H. contortus* (34.9%—larvae + adults) and *Trichostrongylus* spp. (32.6%—adults) of the original inocula were recovered at necropsy from the control lambs.

There appears to be no obvious explanation for the presence of large numbers of 4th-stage *H. contortus* in the control and some treatment groups at the termination of this trial. The results of the small scale test (Table 4) conducted to determine if simultaneous *Trichostrongylus* spp. infections had a reproducible inhibitory effect on the development of *H. contortus* were essentially negative. Substantial numbers of adult *H. contortus* (avg 1,664), and very small numbers of 4th-stage larvae (avg 20), were recovered at necropsy from the two lambs inoculated with all three species of nematodes. Also, substantial numbers of adult worms (avg 1,240) and no 4th-stage larvae were recovered from the two lambs inoculated with comparable numbers of *H. contortus* only. It is also noteworthy that the two lambs with mixed infections harbored larger numbers of adult *Trichostrongylus* spp. (avg 24,380 = 55% of inocula) than the control lambs in the anthelmintic trial (avg 14,470 = 32% of inocula). The results of this supplementary test indicate, therefore, that the concurrent *Trichostrongylus* spp. infection was not a major cause of the inhibited development of *H. contortus* in the anthelmintic trial.
zole. The fact that we obtained with the AH-2 isolate an efficacy of only 65% against adult *H. contortus*, and apparently no activity against inhibited 4th-stage larvae, tends to support the earlier observations, and indicates that the AH-2 isolate may be resistant to thiabendazole at the 50 mg/kg level. Further studies are in progress to compare more precisely the efficacy of thiabendazole against this and other isolates of *H. contortus*. Most reports (see Drudge et al., 1964; Gibson, 1965) indicate that thiabendazole at 50 mg/kg is at least 90% effective against adult *H. contortus* in sheep. Indeed, Gibson (1965) recommends this regimen as the treatment of choice. Conway (1964), on the other hand, reported poor results at this dose level, but improved efficacy at 80 mg/kg. At the dose rate of 44 mg/kg, Drudge et al. (1964) administered six successive drenches at 4-week intervals to naturally infected sheep on pasture with notable lack of success in controlling *H. contortus*. Although fewer *H. contortus* were recovered at necropsy from the principals than from the controls, the residual worm burdens in the treated animals were almost pure infections of *H. contortus*. They concluded that this treatment program apparently selected, from the original population of *H. contortus*, a strain resistant to the drug at this dose level. Attention should be called to the reports of Gibson (1964), Gordon (1964), Reinecke et al. (1962), and Southcott (1963) that thiabendazole at 50 mg/kg body weight is effective against immature *H. contortus*. However, only Reinecke et al., examined digests for the immature stages.

Purified, 2–3 μ phenothiazine given at 550 mg/kg to a group of four lambs removed 99% of adult *H. contortus* (AH-2), but apparently had no effect on inhibited 4th-stage larvae. These results may be compared with those reported by Colglazier et al. (1967) for three other phenothiazine products given at the same dose rate to lambs experimentally infected with another isolate (AH) of *H. contortus* from the same sheep flock. They reported efficacies against adult parasites of 12% for N.F. 10 μ phenothiazine, 40% for N.F. 7 μ phenothiazine, and 75% for purified 6.9 μ phenothiazine. There is a lack of data on the effect of phenothiazine against immature *H. contortus*. However, Gordon (1940) reported that phenothiazine in doses of 600 mg/kg of body weight destroyed immature *H. contortus* 10 and 15 days old. Later, Southcott (1963) reported that phenothiazine in similar doses was less effective against *H. contortus* 12 hr to 7 days old than against parasites 10–28 days old.

A unique finding in this trial was the recovery of substantial numbers of 4th-stage *H. contortus* from lambs of the control, phenothiazine, and thiabendazole groups. These 4th-stage larvae were 1.5–2.5 mm long, and were equivalent in size and development to 4th-stage larvae 4–6 days old, as described by Veglia (1915). For reasons given below, we believe these larvae developed from the original inocula, were inhibited in their development by some factor(s) not now apparent, and were present in all groups of lambs at the time of treatment 28 days postinfection. Therefore, efficacy calculations are given in Table 1 also for activity of the anthelmintics against 4th-stage *H. contortus*. On this basis, only the two tetramisoles showed action against these inhibited larvae.

We cannot be certain the 4th-stage *H. contortus* recovered from the trial lambs came from the original inocula and were inhibited in their development, but the following reasons support this thesis: (1) The animal pens were cleaned regularly. (2) The experiment was conducted in midwinter and the animal quarters were too cold for normal development of infective larvae in the pens. (3) No parasitic larvae of *Trichostrongylus* spp. were recovered from any of the 20 lambs on trial, which strongly supports our contention that reinfection did not occur from pen contamination. (4) The numbers of adult *H. contortus* were low and the numbers of adult *Trichostrongylus* spp. were high in the lambs; therefore, if reinfection had occurred in the pens, substantial numbers of immature *Trichostrongylus* spp. would have been recovered along with immature *H. contortus*, which was not the case. (5) The total numbers of *H. contortus* and *Trichostrongylus* spp. recovered at necropsy from the controls in both instances were about one-third of the original inocula.

**Summary**

In a controlled test with experimental infections of *Haemonchus contortus*, *Trichostrongylus axei*, and *Trichostrongylus colubriformis* in lambs, 1-tetramisole (8 mg/kg), 4-tetrami-
sole (15 mg/kg), thiabendazole (50 mg/kg), and purified, 2–3 μg phenothiazine (550 mg/kg) were all highly effective (94–100%) against adult parasites of all species, except for thiabendazole which was only 65% effective against adult *H. contortus*. The poor results obtained with thiabendazole suggest that the *H. contortus* isolate (AH-2) used in this work may be resistant to the drug at the 50 mg/kg level. Substantial numbers of apparently inhibited 4th-stage *H. contortus* were recovered from all lambs except those treated with tetramisol.

**Acknowledgment**

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**Literature Cited**


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A New Trematode Cephalogonimus sireni sp. nov. (Digenea: Cephalogonimidae) from Florida Mud Eel, Siren lacertina

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In amphibians, a single genus Cephalogonimus Poirier, 1886, of the family Cephalogonimidae, has been recorded mostly from frogs of the genus Rana. From urodeles, only one species, C. amphiuma Chandler, 1923, has been reported from Amphiuma means in Louisiana and subsequently Manter (1938) reported the same species from Siren of Florida. Two mud eels, Siren lacertina, were examined for helminth infection, and one was found to be infected with a new trematode of the genus Cephalogonimus. The worms were fixed under slight coverglass pressure in hot AFA and stained with Semichon’s carmine.

All measurements are in microns, unless otherwise indicated.

Cephalogonimus sireni sp. nov. (Fig. 1)

HOST: Siren lacertina Linnaeus, 1766.
LOCALITY: Lake Munson, Leon County, Florida.
LOCATION: Latter half of intestine.
NUMBER OF WORMS: Five from one host.

1 Present address: Department of Zoology, University of Lucknow, Lucknow (U.P.), India.

Description

Body oval, spinous, small, 1.04–1.44 mm long, and 760–870 wide. Oral sucker sub-terminal, 170 in diameter; ventral sucker very large, 340 in diameter; both suckers have three to six rows of spines on their margins. Pre-pharynx thick and muscular; pharynx 125–130 by 110, very muscular, globular, resembling a sucker; esophagus absent; intestinal cec a terminate almost at posterior end of body. Distance between ceca and posterior end of body 140–150.

Genital pore median and terminal. Testes 230–270 by 200–230, ovoid to spherical, symmetrically opposite, immediately posterior to ventral sucker. Vasa efferentia join in acetabular region to form the vas deferens. Cirrus sac preacetabular, elongated; enclosing elongated seminal vesicle, ejaculatory duct, cirrus and prostatic cells; runs toward left of pharynx and then joins uterus to form hermaphroditic duct that runs dorsal to oral sucker. Ovary submedian, ovoid to lobed, dorsal and to right of ventral sucker, measures 160–170 by 100–110. Laurer’s canal and seminal receptacle present. Vitellaria follicular, extends