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Anthelmintic Activity of Fenbendazole against Experimental *Metastrongylus apri* Infection in Swine

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Key words: swine, internal parasites, lungworms, *Metastrongylus*, anthelmintic, fenbendazole

Introduction

Fenbendazole* (Panacur, Hoechst AG, Federal Republic of Germany) has been reported to be highly effective against gastrointestinal nematodes of swine (Enigk *et al.*, 1974; Kirsch and Duwel, 1975; Batte, 1978; Marti *et al.*, 1978). Batte (1978) demonstrated efficacy against the swine kidney worm, *Stephanurus dentatus*.

The purpose in this present trial was to evaluate the anthelmintic activity of fenbendazole against experimental *Metastrongylus apri* infection in swine.

Materials and Methods

Experimental Pigs: Eighty crossbred pigs (Duroc, Landrace, Yorkshire) were obtained at approximately 6 weeks of age from a primary specific pathogenfree (SPF) herd. Pigs were housed in isolation rooms with concrete floors and fed a 16% crude protein corn-soybean meal diet fortified with minerals and vitamins. The diet was formulated at the University of Nebraska feed mill.

Lungworm Larvae: Lungs of infected pigs were obtained at an abattoir and brought to the laboratory, where adult *Metastrongylus apri* were recovered and placed in physiologic saline for 24 hrs at room temperature. This allowed the lungworms to release numerous eggs into the saline solution. Then the lungworms were minced thoroughly with scissors to release additional eggs. This mixture was poured onto a moistened filter paper in a culture can containing soil.

One hundred earthworms, *Eisenia foetida*, were placed on top of the filter paper in each culture can and covered with soil. Within 48 hrs, the earthworms had ingested the majority of lungworm eggs. Cultures were kept at room temperature and development of the lungworm larvae was checked by dissecting an earthworm. Lungworm larvae reached the infective stage in 3 to 4 weeks following ingestion of *Metastrongylus* eggs by the earthworms.

Preparation of Inoculum: Earthworms were taken from culture cans, washed several times with distilled water, cut into small pieces with scissors, and digested at 37 C for 4 hrs using half-strength artificial gastric juice. The latter was prepared by dissolving 1 g of pepsin in 122 ml of 0.2 N HCl. The suspension containing the lungworm larvae and digestant was centrifuged, and the pellet was washed 4 times in sterile physio-

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* Methyl 5-(phenylthio)-2-benzimidazole carbamate.

logic saline solution (Ferguson, 1971).

The number of infective lungworm larvae present was determined by counting the number of larvae in 9 aliquots of the total material. To establish *Metastrongylus* infection, the inoculum containing 1,500 larvae was administered with a hypodermic syringe connected to a stomach tube. The syringe and stomach tube were rinsed with distilled water to ensure that each pig received the full dose of larvae.

Examination of Feces: Fecal samples from individual pigs were collected daily commencing 25 days after dosing with infective lungworm larvae. Samples were examined microscopically, using a direct centrifugal flotation method. Saturated sodium nitrate was used as the flotation medium.

Administration of Fenbendazole: Exp. I. Forty pigs infected with *Metastrongylus* were individually weighed 24 hrs prior to administration of fenbendazole and randomly assigned to the following treatments: (1) control (n=10); (2) 15 mg fenbendazole/kg body weight (n=10); (3) 20 mg fenbendazole/kg body weight (n=10); and (4) 25 mg fenbendazole/kg body weight (n=10).

The calculated amount of fenbendazole was weighed for each pig and mixed with 0.0454 kg of feed for each of 4.54 kg body weight. The ten pigs in each treatment group were placed in separate rooms and medicated individually by placing the medicated feed in a metal trough. Each of 10 infected, untreated control pigs was given a corresponding amount of the 16% crude protein diet. Following consumption of medicated feed, pigs were maintained on the 16% ration.

Exp. 2. Forty pigs infected with *Metastrongylus* were individually weighed 24 hrs before administration of the fenbendazole and randomly assigned to the following treatments: (1) control (n=10); (2) 2 mg

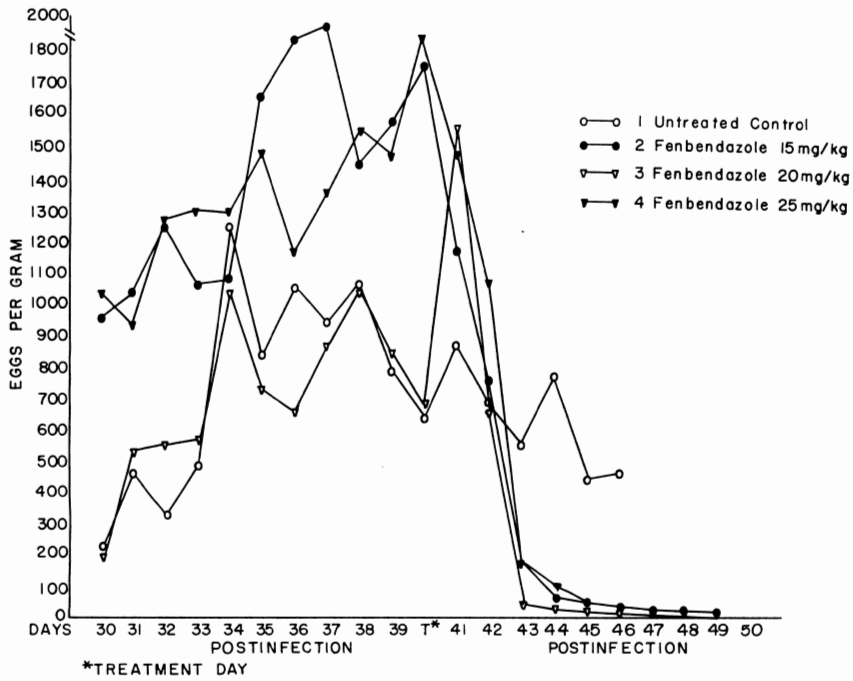

Fig. 1 *Metastrongylus* eggs in feces of experimentally infected pigs before and after treatment with fenbendazole.

fenbendazole/kg body weight once daily for 3 consecutive days (n=10); (3) 3 mg fenbendazole/kg body weight once daily for 3 consecutive days (n=10); and (4) 5 mg fenbendazole/kg body weight once daily for 3 consecutive days (n=10).

The ten pigs in each treatment group were placed in 10 separate rooms. The calculated amount of fenbendazole was weighed for each pig and mixed with 0.0454 kg of feed for each 4.54 kg body weight. The medicated feed was then placed in a metal trough and offered individually to the ten pigs in each treatment group. After consuming the medicated feed, the pigs were maintained on the 16% crude protein diet. Each of the 10 infected, untreated control pigs was given a corresponding amount of the 16% diet.

After treatment, each pig was closely observed for signs of drug toxicity and acceptance of medicated feed.

Necropsy Findings: Pigs were killed in a period 7 to 12 days after treatment with fenbendazole. Lungworm counts were made by opening the bronchial tree and removing the lungworms into a 0.85% saline solution to which 10% glycerol had been added. The lungworms were counted and the counts transformed to the logarithms of X+1 and subjected to an analysis of variance for a completely randomized design. Orthogonal comparisons were used to test the mean differences between the untreated control and dosage levels as well as linear effects of dosage levels. (Snedecor and Cochran, 1967).

Results and Discussion

Exp. I. Problems of acceptance of medicated feed or signs of drug toxicity were

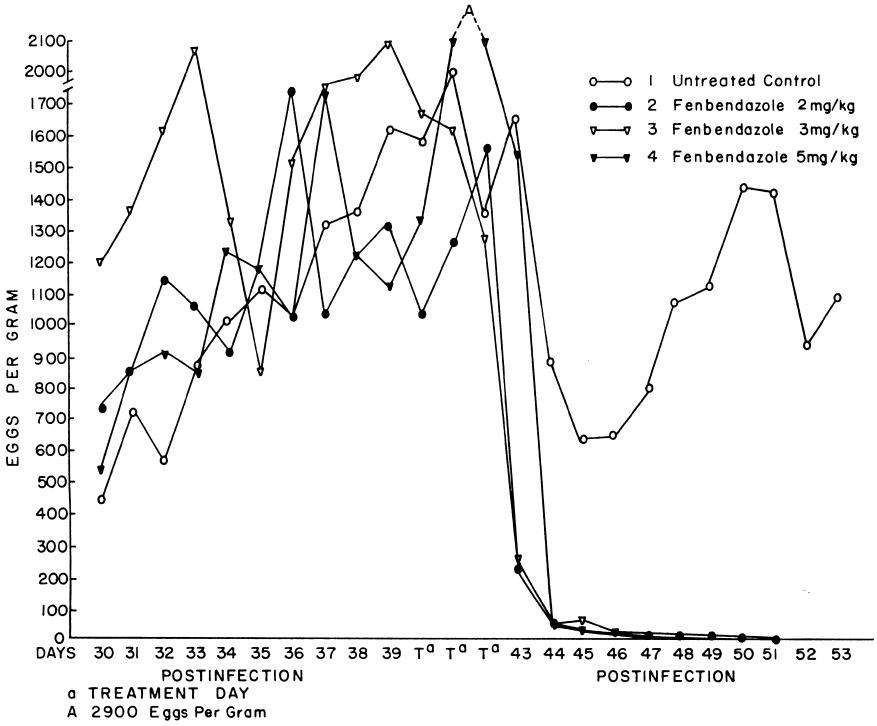

Fig. 2 *Metastrongylus* eggs in feces of experimentally infected pigs before and after treatment with fenbendazole.

Table 1 Anthelmintic activity of fenbendazole against experimental *Metastrongylus* spp. infection in swine

Group No.	Pig No.	Dose (mg/kg)	Lungworms Recovered	Total (mean)	Anthelmintic efficiency (%)
	2-0		878		
	2-4		979		
	3-0		679		
I	4-0		1322		
Unmedicated	7-0	—	1309	855.2*	
controls	4-1		652		
	2-3		956		
	3-1		1084		
	6-0		398		
	7-1		295		
	1-3		141		
	2-7		34		
	2-8		22		
II	4-7		46		
Fenbendazole	5-2	15	0	106.7	87.5
in feed	1-4		0		
	3-6		164		
	3-7		424		
	5-3		35		
	8-1		201		
	1-0		44		
	1-1		66		
	2-1		22		
III	3-2		11		
Fenbendazole	5-0	20	94	77.8	90.9
in feed	1-2		0		
	4-2		64		
	5-1		127		
	8-0		262		
	9-0		88		
	4-8		0		
	6-1		5		
	8-2		6		
IV	10-1		149		
Fenbendazole	11-1	25	2	18.5	97.8
in feed	5-4		23		
	6-2		0		
	7-2		0		
	8-3		0		
	9-1		0		

* The control is statistically different from the fenbendazole application and the dosage of fenbendazole has a significant linear effect at 5% level of significance.

Table 2 Anthelmintic activity of fenbendazone against experimental *Metastrongylus apri* infection in swine

Group No.	Pig No.	Dose (mg/kg)	Lungworms Recovered	Total (mean)	Anthelmintic efficiency (%)
	2-0		1202		
	3-0		1126		
	3-27		236		
I	3-25		840		
Unmedicated	10-0	—	944	1000.7*	—
controls	2-1		1484		
	3-1		1254		
	4-0		851		
	9-0		1117		
	9-24		936		
	2-2		46		
	3-3		9		
	6-21		10		
II	6-23		16		
Fenbendazole	7-0	2	52	22.1	97.8
in feed	1-0		12		
	4-1		56		
	5-0		0		
	8-0		5		
	8-22		15		
	0-1		2		
	4-2		1		
	5-1		3		
III	6-0		3		
Fenbendazole	8-1	3	0	6.6	99.3
in feed	1-20		7		
	1-23		0		
	5-2		2		
	7-1		0		
	9-1		48		
	2-3		0		
	3-3		0		
	5-21		0		
IV	5-24		0		
Fenbendazole	7-2	5	0	0.1	99.9
in feed	4-3		1		
	6-1		0		
	7-3		0		
	8-2		0		
	11-0		0		

* The control is statistically different from the fenbendazole application and the dosage of fenbendazole has a significant linear effect at 5% level of significance.

not observed. The 30 pigs consumed all of the feed within one hour. Loss of appetite was not observed following treatment, and consumption of feed and water remained normal throughout the experimental period.

Clinical signs resulting from lungworm infection were coughing, beginning at 10 to 12 days postinfection, and rapid shallow breathing. Untreated controls developed a loose, husky cough which persisted until termination of the experiment 49 days postinfection.

The number of *Metastrongylus* eggs per gram of feces for each experimental group is shown in Figure 1. Lungworm eggs were observed in feces of 40 pigs within 28 days after oral dosing with approximately 1,500 3rd-stage larvae. Within six days after receiving fenbendazole, a marked reduction in the number of lungworm eggs per gram of feces was observed in the 30 medicated pigs. Lungworm eggs were observed in the feces of 10 untreated control pigs (Group 1) from Day 28 postinfection until termination of the experiment.

Fenbendazole exhibited excellent anthelmintic activity against swine lungworms when administered in feed at levels of 15, 20 or 25 mg/kg. Percentage of efficacy was determined by comparing worm burdens of treated pigs with those of untreated controls. When administered in feed, anthelmintic efficacy was 87.5% at 15 mg/kg; 90.9% at 20 mg/kg; and 97.8% at 25 mg/kg. Orthogonal comparisons between dosage levels and the control indicated significant differences at the 5% level as well as a linear effect due to dosage level (Table I).

While removing lungworms from two pigs, it was observed that a number of worms were brown instead of the characteristic white color (Pig 5-1–127 worms; pig 9-0–88 worms, Group III). In addition, most of the worms from these two pigs were females with only a small number of male worms present.

Exp. II. Signs of drug toxicity or problems of acceptance of medicated feed were not observed. On each treatment day, the 30 pigs consumed all of the medicated feed within one hour. Loss of appetite was not observed and consumption of feed and water remained normal.

Clinical signs resulting from lungworm infection were similar to those observed in Exp. I. These included coughing, beginning at 10 to 12 days postinfection, and rapid shallow breathing. The untreated controls (Group I) developed a loose, husky cough which persisted until termination of the experiment. Pig 2-1 (Group I) died 40 days postinfection and 1474 lungworms were recovered (Table 2).

Lungworm eggs were observed in the feces of 40 pigs within 28 days after oral dosing with approximately 1500 3rd-stage larvae. After receiving fenbendazole three times in feed, a marked reduction in the number of lungworm eggs per gram of feces was observed in the 30 treated pigs. Lungworm eggs were observed in feces of each of the 10 untreated control pigs from Day 28 postinfection until termination of the experiment 54 days postinfection (Figure 2).

Fenbendazole exhibited excellent anthelmintic activity when administered in feed once daily for three consecutive days at levels of 2, 3, or 5 mg/kg. Anthelmintic efficacy was 97.8% at 2 mg/kg; 99.3% at 3 mg/kg; and 99.9% at 5 mg/kg. Orthogonal comparisons between dosage levels and the control indicated significant differences at the 5% level as well as a linear effect due to dosage level (Table 2).

Fenbendazole was shown by Batte (1978) to be highly effective against *Ascaris suum* and *Trichuris suis* of pigs at single doses of 3 to 25 mg/kg or multiple doses of 3 to 5 mg/kg for three days. Consistently higher efficacy was obtained by the multiple daily dosing regimen than by single dosing, even when the total drug intake was comparable.

In the present study, fenbendazole exhibited considerably higher efficacy against swine lungworms when administered once daily for three days in the feed than by single dosing, even though the total drug intake was lower.

Ferguson (1981) reported similar results with albendazole. When albendazole was fed continuously to pigs for 5 days at 10 ppm, 20 ppm or 30 ppm, the efficacy against *Metastrongylus apri* was higher than by single dosing, and yet less drug was consumed by the individual pig.

Prichard *et al.* (1978) investigated the effects of prolonged administration of the benzimidazole anthelmintics — cambendazole, fenbendazole, oxfendazole, and thiabendazole on parasitic helminths in cattle and sheep. Their observations suggest that the spectrum and effectiveness of benzimidazoles may be improved by extending the period during which parasites are exposed to toxic concentrations.

Summary

Exp. I. Three groups of 10 pigs experimentally infected with 3rd-stage larvae of *Metastrongylus apri* were treated once in feed with fenbendazole at levels of 15, 20 or 25 mg/kg body weight at 40 days postinfection. Anthelmintic efficacy was 87.5, 90.9, and 97.8% respectively when compared on the basis of post-mortem examination worm counts, with 10 infected, untreated control pigs. Orthogonal comparisons between dosage levels and the control indicated significant differences at the 5% level as well as a linear effect due to dosage level. Exp. II. Three groups of 10 pigs experimentally infected with 3rd-stage larvae of *Metastrongylus apri* were treated in feed with fenbendazole at levels of 2, 3, or 5 mg/kg body weight once daily for 3 consecutive days commencing at 40 days postinfection. Anthelmintic efficacy was 97.8, 99.3, and 99.9% respectively when compared on the basis of post-mortem examination worm counts, with 10 infected, untreated control pigs. These mean reductions from the control values were significant ($P<.05$) and there was a linear effect due to dosage level.

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豚肺虫 *Metastrongylus apri* 実験感染豚に対する fenbendazole の駆虫効果

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実験 1: 豚肺虫の第3期幼虫を40頭の豚に感染させ，これを10頭ずつ4群に分け感染後40日目に fenbendazole を体重 kg 当りII～IV群にはそれぞれ15，20，25 mg ずつ経口的に投与し，その駆虫効果を検討した．すなわち対照（I群）の無処置群の虫体回収率と処置群のそれとを比較した結果，駆虫効果はそれぞれ 87.5，90.9 および 97.8% で，投与量の増加とともに駆虫効果の増大が認められた．

実験 2: 実験1と同様に豚肺虫の第3期幼虫を感染させた豚に，感染後40日目に fenbendazole を体重 kg 当り 2，3，5 mg ずつ3日間各々各群に投薬した．その結果駆虫効果はそれぞれ 97.8，99.3，99.9% で本実験でも投薬量の増加とともに効果の増大することが確認された．