

Anthelmintic Activity of Injectable Ilevamisole against Experimental *Metastrongylus apri* Infection in Swine

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Introduction

The anthelmintic activity of levamisole against swine lungworms administered in the feed or drinking water to pigs has been reported by many investigators (Dixon, 1969), (Ferguson, 1971), (Ferguson and White, 1975), (Lee and Kim, 1968), (Lindquist *et al.*, 1971), (Poeschel and Emro, 1972), (Smith, 1972), (Ueno *et al.*, 1967), (Walley, 1967), (Wikerhauser and Zukovie, 1968), (Wood and Ramirez-Miller, 1966), but published evidence of the efficacy of subcutaneously administered levamisole against larval or adult stages is lacking in the United States. The purpose in the present trial was to evaluate the anthelmintic activity of injectable levamisole against experimental *Metastrongylus apri* in swine.

Materials and Methods

Experimental Pigs: Thirty-two crossbred

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pigs (Duroc, Landrace, and Yorkshire) were obtained at six weeks of age from a primary specific pathogen-free (SPF) herd. Pigs were randomly divided into four groups of eight pigs each. Each group was housed in an isolation room with concrete floor and allowed a balanced diet ad libitum.

Lungworm Larvae: Adult *Metastrongylus apri* were recovered from the lungs of infected pigs and placed in physiologic saline for 24 hours 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 filter paper in a culture can.

One hundred earthworms, *Eisenia foetida*, were placed on the filter paper in each culture can and covered with soil. Cultures were kept at room temperature and development of the lungworm larvae was checked at frequent intervals by dissecting an earthworm. The infective stage was reached 3 to 4 weeks following ingestion by 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 hours. Half-strength artificial gastric juice, made by dissolving 1 g of pepsin in 120 ml of distilled water and 2 ml of concentrated hydrochloric acid, was used

to release lungworm larvae from earthworm tissues. The suspension containing the lungworm larvae and digestant was centrifuged and the pellet was washed several times in sterile physiologic saline solution.

The number of infective lungworm larvae present was determined by counting the number of larvae in nine aliquots of the total material. To establish *Metastrongylus* infection, the inoculum containing 1500 lungworm larvae was administered through a stomach tube and a hypodermic syringe. The stomach tube was rinsed with distilled water to assure the full dose of larvae per pig.

Examination of Feces: Fecal samples from individual pigs were collected daily for 13 days starting at 25 days postinfection and ending at 37th day postinfection. Samples were examined microscopically, using a direct centrifugal flotation method. Saturated sodium nitrate was used as the flotation medium.

Administration of Levamisole: Levamisole (6.8% solution) was injected one time subcutaneously in the flank area at a rate of 6 mg/kg of live body weight. Following treatment, the pigs were closely observed for signs of drug toxicity.

Necropsy Findings: Pigs were euthanized 35 to 38 days postinfection. The lungworm counts were made by opening the bronchi and removing the lungworms into a 0.85% saline solution to which 10% glycerol had been added. The lungworms could be kept in this solution for several hours without rupturing. Lungworm counts were transformed to the logarithms of $X+1$ and subjected to an analysis of variance.

Results and Discussion

In the present study, levamisole exhibited excellent anthelmintic activity against swine lungworms when injected subcutaneously at a rate of 6 mg/kg of live body weight. Percentage of efficacy was

determined by comparing worm burdens of treated pigs with those of untreated controls. When injected subcutaneously at 5 days postinfection, the anthelmintic efficacy was 99.98%; at 15 days postinfection, it was 100%; and at 30 days postinfection, it was 99.98% (Table 1). The results of this study are in agreement with Oakley (1974a, 1975b). Data from these studies indicate that injectable levamisole would be effective against *Metastrongylus apri* at a lower dosage level than 7.5 or 6 mg/kg.

Numbers of *Metastrongylus* eggs per gram of feces of pigs in each experimental group are shown in Table 2. Lungworm eggs were observed in the feces of pigs in Group I (untreated controls) and Group IV (levamisole 30 days postinfection) within 28 days after oral dosing with 1500 thirdstage infective larvae.

Clinical signs resulting from lungworm infection were coughing, beginning at 10 to 12 days postinfection, rapid shallow breathing, and loss of appetite. The unmedicated controls (Group I) developed an unproductive, husky cough which persisted until termination of the experiment.

Five minutes after receiving the subcutaneous injection of levamisole, each of the eight pigs in Group IV (30 days postinfection) started coughing. Within 20 minutes, each pig had coughed up varying amounts of mucus that contained numerous mature lungworms. During this 20 minute period, the pigs made frequent trips to the water bowl. Coughing persisted for approximately 45 minutes.

Lungworm eggs were observed in the feces of each of eight control pigs (Group I) from Day 28 until termination of the experiment (Table 2). A marked reduction in the number of lungworm eggs per gram of feces was observed in the eight pigs from Group IV following injection of levamisole (Table 2). Lungworm eggs were not observed in the feces of pigs from Groups II and III.

Table 1 Anthelmintic activity of levamisole against experimental *Metastrongylus apri* in swine

Group	Pig No.	Dose (mg/kg)	Lungworms Recovered	Total (mean)	Anthelmintic Efficiency (%)
I Unmedicated Controls	1-28		1377	824 ^{a*}	
	1-34		822		
	2-41		450		
	2-43	—	1526		
	3-35		184		
	5-43		1061		
	7-43		109		
	8-16		1064		
II Levamisole 5 days PI	1-10		0	0.125 ^b	99.98
	1-11		0		
	2-11		0		
	3-11	6	0		
	4-11		1		
	5-11		0		
	8-11		0		
	2-12		0		
III Levamisole 15 days PI	1-12		0	0 ^b	100
	3-12		0		
	4-12		0		
	5-12	6	0		
	6-12		0		
	7-12		0		
	6-11		0		
	9-11		0		
IV Levamisole 30 days PI	11-11		0	0.125 ^b	99.98
	12-13		0		
	4-11		0		
	6-14	6	0		
	7-14		0		
	8-14		0		
	9-14		0		
	10-14		1		

PI: Postinfection

* Means with different subscripts are significantly different (P .01).

Expelled lungworms were collected from the expectorated mucus and placed in physiologic saline for 24 hours at room temperature. Then the lungworms were minced thoroughly with scissors. This mixture was placed on filter paper in a culture can containing soil. Fifty earthworms, *Eisenia foetida*, were placed in the culture can on top of the filter paper. The culture

can was 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 by earthworms.

Infective lungworm larvae were collected and administered to two pigs. Fecal samples were collected daily and lungworm

Table 2 *Metastrongylus* eggs in feces of experimentally infected pigs before and after treatment with levamisole

Group	Pig No.	Dose (mg/kg)	Days after Infection												
			25	26	27	28	29	30	31	32	33	34	35	36	37
I Untreated Controls	1-28		0	0	0	66*	52	64	172	514	216	768	1024	+	
	1-34		0	0	0	2	2	28	8	58	194	170	360	+	
	2-41		0	0	0	8	6	48	178	292	784	1054	2656	+	
	2-43	—	0	0	0	0	6	0	12	16	192	216	304	+	
	3-35		0	0	0	6	2	0	10	0	4	0	12	+	
	5-43		0	0	0	6	24	66	122	340	320	670	1792	+	
	7-43		0	0	0	6	6	6	10	64	66	98	288	+	
	8-16		0	0	0	10	4	18	58	240	326	504	528	+	
II Levamisole 5 days PI	1-10		0	0	0	0	0	0	0	0	0	0	0	0	+
	1-11		0	0	0	0	0	0	0	0	0	0	0	0	+
	2-11		0	0	0	0	0	0	0	0	0	0	0	0	+
	3-11	6	0	0	0	0	0	0	0	0	0	0	0	0	+
	4-11		0	0	0	0	0	0	0	0	0	0	0	0	+
	5-11		0	0	0	0	0	0	0	0	0	0	0	0	+
	8-11		0	0	0	0	0	0	0	0	0	0	0	0	+
	2-12		0	0	0	0	0	0	0	0	0	0	0	0	+
III Levamisole 15 days PI	1-12		0	0	0	0	0	0	0	0	0	0	+		
	3-12		0	0	0	0	0	0	0	0	0	0	+		
	4-12		0	0	0	0	0	0	0	0	0	0	+		
	5-12	6	0	0	0	0	0	0	0	0	0	0	+		
	6-12		0	0	0	0	0	0	0	0	0	0	+		
	7-12		0	0	0	0	0	0	0	0	0	0	+		
	6-11		0	0	0	0	0	0	0	0	0	0	+		
	9-11		0	0	0	0	0	0	0	0	0	0	+		
IV Levamisole 30 days PI	11-11		0	0	2	0	6	14	0	0	0	0	0	+	
	12-13		0	0	0	8	44	164	0	0	0	0	0	+	
	4-14		0	0	0	2	18	70	0	2	0	0	0	+	
	6-14	6	0	0	0	4	14	36	0	0	0	0	0	+	
	7-14		0	0	0	10	18	72	0	0	2	0	0	+	
	8-14		0	0	0	4	6	12	0	0	0	0	0	+	
	9-14		0	0	0	6	4	48	0	0	4	0	0	+	
10-14		0	0	0	14	20	68	0	0	2	0	0	+		

* : Number of worm eggs per gram of feces in individual samples.

+ : Pig killed

PI: Postinfection

eggs were observed in the feces at 28 days postinfection. Apparently, levamisole treatment did not prevent the normal development of lungworm eggs from female worms following expulsion from the lungs.

Toxicosis due to the drug was not observed in the pigs at a treatment level of 6 mg/kg.

Summary

Three groups of eight pigs experimentally infected with 3rd-stage larvae of *Metastrongylus apri*, were injected subcutaneously with levamisole (6.8% solution) at a rate of 6 mg/kg live body weight at 5, 15,

and 30 days postinfection.

Anthelmintic efficiency was 99.98, 100, and 99.98% respectively when compared on the basis of post-mortem examination worm counts, with one group of eight infected, untreated control pigs.

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豚の *Metastrongylus apri* 実験感染に対する Levamisole 注射の駆虫効果

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豚肺虫 *Metastrongylus apri* を実験的に感染させた幼豚を用い、levamisole の皮下注射による駆虫効果を検討した。実験豚は6週齢の3元交配(デュロック・ランドレース・ヨークシャー)豚32頭で、一群を8頭として無作為に4群に分けた。豚への実験的感染にはミミズ *Eisenia foetida* で培養して得た第三期子虫を胃管を用いて投与した。4群のうちI群は無投薬対照群で、II・III・IVの3群は投薬群である。投薬群に

は実験的感染後II群は5日、III群は15日、IV群は30日に、各々の実験豚に levamisole 6.8% 液を 6 mg/kg として腹側に1回皮下注射した。感染後35~38日に安楽死させて剖検し、投薬群の駆虫効果を気管支から回収された肺虫数について、無投薬対照群と比較検討した。駆虫効果はII群が99.98%、III群が100%、IV群が99.98%であった。また、薬物による副作用は観察されなかった。