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BIONOMICS OF LUNGWORMS OF SWINE

by

Himansu Gopal Sen

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Presented to the Faculty of

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In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Department of Zoology

Under the Supervision of Professors Harold W. Manter

and George W. Kelley, Jr.

Lincoln, Nebraska

May, 1960

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TITLE

Bionomics of Lungworms of Swine

BY

Himansu Gopal Sen

APPROVED	DATE
H. W. Manter	May 26, 1960
George W. Kelley Jr.	May 26, 1960
Thomas B. Thorso	May 26, 1960
Robert M. Wotton	May 26, 1960
George A. Young	May 26, 1960
Harold J. Bell	May 26, 1960

SUPERVISORY COMMITTEE

GRADUATE COLLEGE UNIVERSITY OF NEBRASKA

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CHAPTER I

INTRODUCTION

The lungworms of swine belong to the genus Metastrongylus, family Metastrongylidae, Order Strongyloidea, Class Nematoda of the Phylum Nemathelminthes. Schwartz and Alicata (1934) determined that midwestern pigs are usually parasitized with a mixed population of two species, M. pudendotectus and M. apri. A third species, M. salmi, occurs but is rarely found in this part of the United States. The distinguishing features of the three species are presented in Table 1.

Details of the life cycle of Metastrongylus spp. were presented by Hobmaier and Hobmaier in 1929 and by Schwartz and Alicata in 1934. The embryonated lungworm eggs deposited by the female lungworm in the bronchioles of the lungs are coughed up by the swine and swallowed. The eggs are shed in the feces. After reaching the soil the embryonated eggs must be ingested by an earthworm in which the first stage larvae hatch from the eggs. In the earthworm the larvae undergo two molts and migrate into the wall of the esophagus and heart. This third stage is usually attained in three to four weeks following the ingestion of embryonated lungworm eggs by earthworms. The larvae are liberated from the earthworm tissue when ingested by swine. They penetrate the intestinal

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TABLE 1

THE DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS OF METASTRONGYLUS APRI, METASTRONGYLUS PUDENDOTECTUS AND METASTRONGYLUS SALMI

Species	Length	Bursa	Spicule length	Vagina Length
M. apri	Male: up to 25 mm	Small	3.9 to 5.5 mm	
	Female: up to 60 mm			Over 2.0 mm
M. pudendotectus	Male: 16 to 18 mm	Large	1.4 to 1.7 mm	
	Female: 19 to 37 mm			1 mm
M. salmi	Male: 17 to 18 mm	Very small	2.1 to 2.4 mm	
	Female: 30 to 40 mm			1.6 mm

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wall and migrate to the lungs via the lymphatics and blood stream. After undergoing two further molts, the larvae become adult lungworms in the bronchioles of the lungs, usually about four weeks post ingestion.

Metastrongylus spp. in the lungs interfere with breathing. In early stages the infection is characterized by a cough due to irritation in the bronchi and bronchioles produced by the arrival of the wandering worms in the lungs. The macroscopic changes of the lungs include vesicular emphysema and consolidation, most commonly found in the diaphragmatic lobes of lungs. The microscopic changes include chronic bronchitis with mucoid metaplasia of the bronchial epithelium and peribronchial lymphoid hyperplasia associated with eosinophil reaction (Dunn et al. 1955; and Mackenzie, 1959).

The bionomics of Metastronylus spp. are obscure. The incidence of the parasite in areas as yet unsurveyed, the effects of age resistance upon the parasite, its inhibited development in an immune host, the cellular response of the latter to parasite attack, rational control measures of the parasite with suitable anthelmintics, the possible transmission of swine influenza virus by the parasite and its physiological and nutritional requirements in vitro are not completely understood.

The present study was undertaken to answer some of these questions.

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Materials and Methods

Growing Infective Larvae: Infective Metastrongylus larvae were cultured in the laboratory. Lungs of pigs were obtained at an abattoir and brought to the laboratory where adult Metastrongylus spp. were recovered from the bronchi. The worms were kept overnight in distilled water at room temperature to release numerous eggs. The worms were then minced thoroughly with scissors to release any eggs which had not been laid. This mince usually contained a mixture of thin shelled eggs and larvae which had just emerged from the eggs as well as thick shelled embryonated eggs. The egg suspension consisting of both oviposited eggs and those released by mincing was mixed into a medium of equal parts of sphagnum moss and soil kept in one-pound coffee cans perforated on both ends. The earthworms were dug in the back yard of the cattle barn close to the laboratory, an area known to have been free of contamination by swine feces for many years. One hundred mature earthworms, Eisenia foetida, were placed in each of the one-pound culture cans. These cultures were kept at room temperature and development of larvae was observed by dissecting the earthworms at frequent intervals. The larvae usually reached the infective stage in three to four weeks following ingestion by the earthworms.

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Preparation of inoculum: Several earthworms were taken from the culture to prepare the inoculum. These earthworms were washed several times in distilled water, cut into small pieces with a razor blade, then subjected to digestion at 37°C for four hours. Half-strength artificial gastric juice, made by dissolving one gram of pepsin in 120 ml of distilled water and two ml of concentrated hydrochloric acid, was used to release larvae from earthworm tissue. The suspension containing larvae and digestant was centrifuged and washed several times in sterile physiological saline. The number of infective larvae present was determined by counting the number of larvae in aliquots of the total material.

Mode of Inoculation: To establish Metastrongylus infection the inoculum prepared in the above manner was given to pigs by gavage. The proper volume to yield the desired number of larvae was introduced into the stomach tube by means of a hypodermic syringe.

Experimental Animals: All pigs used in these experiments were obtained by hysterectomy according to the techniques described by Young et al. (1955). The pigs were delivered and separated from embryonic membranes within a sterile hood two to four days before expected parturition. They were reared during the early part of their lives in individual

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isolation units on modified cows' milk. No colostrum or antibiotics were fed to any of these pigs. At three to four weeks of age, unless otherwise stated, the animals were transferred to isolation units containing several pigs or to closed rooms having concrete floors. Their diet at this time consisted of a commercial feed supplemented with vitamins, minerals and antibiotics. They were kept under careful supervision to preclude any extraneous infections with Metastrongylus spp.

Fecal Examination: The presence of Metastrongylus eggs in fecal specimens was determined by the modified salt flotation technique (Stoll, 1930).

The fecal specimen was collected from the rectum of the pig and placed in a small clean specimen jar. Ten grams of the feces were weighed into a 450 ml shaking jar to which 300 ml tap water was added. Several solid glass beads were put in the jar and shaken thoroughly to free the eggs from the fecal bolus and distribute them evenly throughout the water mixture. When the mixture had been sufficiently shaken, 15 ml were poured promptly into a centrifuge tube. This tube, containing one-half gram of the original specimen, was then centrifuged for one minute at 1,000 r.p.m., settling the debris and eggs at the bottom of the tube. The supernatant was decanted and discarded. About 10 ml saturated sodium nitrate solution (specific gravity 1.370) was added to the

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remaining plug in the tube. The eggs were again brought in suspension by stirring the mixture with a stick. The tube was once more placed in the centrifuge and carefully filled with sodium nitrate until the solution raised a meniscus over the lip of the tube. A cover glass was gently dropped on to this surface and the preparation was centrifuged for one minute at 1,000 r.p.m. The cover glass was lifted carefully with forceps, put on a slide and examined with the aid of a microscope.

Since one-half gram of feces had been examined, the number of eggs per gram of feces (EPG) was determined by doubling the total number of eggs found on the cover slip.

Procedure of Necropsy: Pigs were anesthetized prior to killing by injecting from five to 10 ml of a solution of sodium pentobarbital intraperitoneally. While anesthetized, the brachial arteries were severed to exsanguinate the animal. After death the chest cavity was opened and the lungs were removed, opened with scissors, and thoroughly examined for the parasites. The worms were removed, counted, and killed immediately in hot A.F.A.[1] to prevent rupturing. Twenty-five worms of each sex were measured to evaluate the characteristics of the population.

---

[1]Formalin 40 per cent, 65 ml; alcohol 50 per cent, one liter and glacial acetic acid 25 ml.

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CHAPTER II

INCIDENCE OF LUNGWORM INFECTION IN SWINE IN NEBRASKA

Metastrongylus apri and *M. pudendotectus* are found distributed throughout the United States. Morgan and Hawkins, (1949) reported lungworms in 31 per cent of 1,000 Michigan pigs. Spindler (1934) recovered *Metastrongylus* from 69 per cent of 348 animals examined in the Southern United States. Sullivan and Shaw (1953) found 52 per cent of infection in 518 pigs from the Willamette Valley of Oregon, and Goldsby and Todd (1957) reported 66 per cent of 101 Wisconsin pigs infected.

No observations have been made of the incidence of swine lungworm infection in Nebraska. The survey reported herein was conducted to determine the parasitic burden of *Metastrongylus spp.* to Nebraska swine producers.

Methods of Survey

Observations on the occurrence of *Metastrongylus spp.* in the lungs of slaughter house swine was started in November, 1958 and conducted through each of eleven successive months at the Hormel packing plant at Fremont, Nebraska. Every fifth animal on the production line was examined until 100 observations had been made. Thus a 20 per cent sample of 500 pigs

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was examined. The caudal end of the diaphragmatic lobe of each lung was excised about two inches from the distal tip. Compression of this piece of lung by the left hand extruded the lungworms from the bronchioles. On occasion the apical and cardiac lobes were excised and examined for the parasites. The whole procedure, excising the tips of the lungs, squeezing the cut surfaces and inspection for worms, did not involve more than 45 seconds for each pig. Lungs were considered positive when a single or several adult worms were seen by the naked eye. This superficial method of examining lungs for Metastrongylus spp. possibly missed some infections, particularly when immature worms were involved, but the quickness of the method permitted a greater number of observations to be made than would otherwise have been possible by more tedious laboratory examinations. About 50 per cent of the pigs slaughtered came from within a 50 mile area of Fremont; 35 per cent from a radius of 50-100 miles and 15 per cent from a 100-200 mile area.

Results: Lungworms were found in 19.5 per cent of the pigs examined during the 12 month period. Monthly incidence is presented in Graph I.

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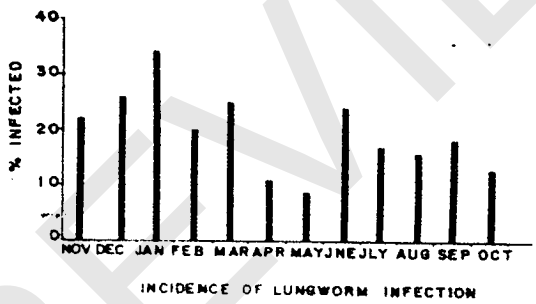

Graph 1. The monthly incidence of swine lungworm infection of Nebraska pigs from November, 1958, to October, 1959.

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Discussion

The incidence of nearly 20 per cent was lower than that found in Michigan, Southern United States, Oregon and Wisconsin, where 31, 69, 52 and 66 per cent respectively have been reported (Morgan and Hawkins, 1949; Spindler, 1934; Sullivan and Shaw, 1953 and Goldsby and Todd, 1957). This difference perhaps can be explained by different husbandry practices which occur in the middle west. Many pigs in Nebraska are reared on concrete floors limiting their ingesting of earthworms. The pigs in Michigan, Oregon and Wisconsin, where a higher incidence of infection has been recorded, are probably raised out of doors and under this condition the earthworms containing the infective larvae accumulate in the soil and are easily accessible to the pigs.

The lungworms in the naturally infected pigs can usually be found in the distal part of the diaphragmatic lobe of the lung. However, in early infections all of the bronchioles contain worms. Dunn et al. (1955) from observations of natural infection of pigs in Great Britain also have found that the parasites locate in the caudal part of the diaphragmatic lobes. The question obviously arises as to why the diaphragmatic lobe more often harbors the parasites than other parts of the lungs. An explanation is difficult because the metabolism and physiology of Metastrongylus are poorly

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understood. From the existing information one might postulate that live or dead worms are more easily expelled from the bronchi in the apical and cardiac lobes of the lungs by coughing than from the more caudal part of the diaphragmatic lobe. The pigs that are not severely infected may get rid of many worms by coughing but it is probable that a light residual infection is retained in the caudal tips of the lungs.

Further analysis of the data reveals that the incidence of infection is lower in April and May than other months. This may be because (1) earthworms are not accessible during the winter season or (2) that fewer pigs killed in these two particular observation periods happened to be infected because they came from locations where earthworms containing the infective larvae were not readily available. The first possibility can be ruled out as experience in the laboratory has shown that the infection is retained in the pigs for several months following infection and season has no influence on limiting the infection. This observation is in agreement with the findings of Lewis (1926) who did not encounter a seasonal variation in frequency of infection with lungworms in Welsh pigs. But winter in England is mild and earthworms are more available than in Nebraska. Further studies of pigs and earthworms on various farms in Nebraska may explain some of these questions about the incidence of infection.

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CHAPTER III

IMMUNITY TO SWINE LUNGWORM

AGE IMMUNITY TO LUNGWORM INFECTION IN SWINE

Ruminants demonstrate a distinct age immunity to lungworms. Kauzal (1934) found that fewer Dictyocaulus filaria developed in lambs five and one-half to seven months old than in younger animals. Porter and Cauthen (1942) indicated age resistance to D. viviparus in calves.

The present study was designed to determine whether age immunity to Metastrongylus spp. exists in pigs.

Materials and Methods

Six pigs three to 20 weeks old were each fed 3,000 Metastrongylus larvae. Of these pigs, two were used in each of three groups of three, 13 and 20 weeks of age respectively. All pigs were killed 20 to 23 days post infection.

Results: An average of 914 worms, measuring 15.0 mm, were recovered from the lungs of the three-week-old pigs; 182 worms, measuring 8.2 mm, were found in the 13-week-old pigs; and 376 worms, measuring 13.9 mm, were in the 20-week-old pigs. Areas of consolidation and bronchopneumonia were more extensive in the youngest (three-week-old) pigs.

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