STUDIES ON *ASCARIDIA COLUMBAE*: EXPERIMENTAL LIFE CYCLE IN PARENTERALLY INFECTED PIGEONS, AND FACTORS AFFECTING THE OVIPOSITION *IN VITRO*

by

ROY DANIEL MELENDEZ,

DVM., Universidad Centro Occidental, 1970

Barquisimeto, Venezuela

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCES

Department of Infectious Diseases

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

Approved by:

William D. Lindquist

Major Professor

TABLE OF CONTENTS

Page

LISTS OF TABLES, GRAFTS, AND PLATES

TABLES

GRAPHS

PLATES

I. INTRODUCTION AND LITERATURE REVIEW

Ascaridia columbae (Gmelin 1790) Travassos 1913, is a common nematode of the small intestine of wild and domestic pigeons (Columba livia domestica), whose life cycle has been occasionally studied. Research on the life-history of domestic fowl nematodes, has been heavily concentrated on the biological system of Ascaridia galli in chickens. This is explained by the importance of chickens as a source of food for man, and the fact that it is easier to raise baby chicks in the laboratory than squabs. Ascaridia columbae is the only ascarid so far found in the domestic pigeon in North America, (Wehr, 1971). The adult parasite was described by Irwin-Smith (1920), by Cram (1927), and by Mozgovoi (1953). Further descriptions on the morphology of immature stages and adult Ascaridia columbae worms were not found in the literature until the last decade. Wehr and Hwang (1964) published a well documented research, not only on the morphology of this interesting nematode, but also on its life-cycle. In their experiments, pigeons were orally infected, using single or multiple doses of embryonated A. columbae eggs. Their statements and conclusions represented the most extensive information available on the biology of this worm; therefore, those were kept in mind during the development of this research as a point of reference and comparison.

Bedel (1902) reported finding Ascaridia columbae larvae in the liver of naturally infected pigeons. Hwang and Wehr (1958), and Wehr, and Hwang (1959), published their observations on the life-cycle and early development of this worm in experimentally infected pigeons. Wehr and

Shalkop (1963) studied the histopathological lesions caused by migrating Ascaridia columbae larvae in the liver of orally infected pigeons. Lindquist, (1963) reported that squabs acquired infections of Ascaridia columbae and Capillaria obsignata from their parents during the feeding process, causing an early infection in squabs, which undoubtedly accounts for the difficulty of obtaining pigeons free of Ascaridia for experimental purposes. Wehr and Hwang (1964) stated that the histotropic phase was not an essential step for completing the development of Ascaridia columbae. Furthermore, they found that many second larval stage (L2) migrated to the liver and few to the lungs, where they were trapped; tracheal migration, and thereby a patent infection in the small intestine of pigeons with worms coming from extraintestinal tissues was prevented. These observations were important when planning this research, since it led to the question, what would be the fate of infective Ascaridia columbae larvae artificially hatched and injected intravenously in susceptible pigeons? The parenteral route has been used before in experimental infections using other ascarids in normal and abnormal hosts, (Arean and Crandall, 1962; Bindseil, 1970a; Copeman and Gaafar, 1972). It has been used in experimental research with other helminths, Gemmell (1969), Froyd and Round (1959), and Urquhart (1965), and even in successful infections of chickens with Eimeria sp. (Sharma and Reid, 1962). As far as Ascaridia columbae is concerned, experimental infection through the parenteral route has advantages for the parasite. It eliminates obstacles to migration such as the gut barrier and the lymphoid tissues of the liver, whose protective immune mechanisms otherwise would mount a strong and efficient immune

response, trapping the immature stages, and preventing them from further migration. This is probably the first investigation carried out where pigeons were intravenously infected with L2 A. columbae, so that the life cycle started with the infective larvae placed in the host's lungs.

In addition to the studies carried out on the life cycle of A. columbae, investigations were also oriented to ascertain some factors which could affect the oviposition of female A. columbae "in vitro." The main point to be assessed was whether the secretions and excretions of male A. columbae had positive or negative effects on the total egg output of female A. columbae.

The objectives of the present study were: (1) To ascertain the routes of migration, and the fate of infective A. columbae larvae in intravenously infected pigeons, (2) To compare the length and size of A. columbae populations recovered from orally and parenterally infected pigeons, (3) To study the lesions caused by migrating larvae in those infected birds, and (4) to investigate some intrinsic and extrinsic factors which might have modified the oviposition of female A. columbae "in vitro."

II. MATERIALS AND METHODS

1) Pigeons:

Twenty five 5-7 week old birds were bought from a large pigeon plant in the Eastern United States. They were divided randomly into two groups, one having 12 birds which arrived first, and were used in experiment No. 1 (oral infections), and the second group utilized in parenteral

infections with L2 stage of A. columbae. Birds were kept in cages (3 or 4 pigeons per cage) for several days, and fed ad libitum, Purina pigeon chow* plus fresh water. All birds were screened for gastro-intestinal parasites by means of daily fecal tests during one week using a salt flotation technique. Many pigeons had slight infections with Eimeria labbeana, two birds used in experiment No. 1 had Capillaria sp., and four birds from experiment No. 1 (pigeons 401, 402, 403, and 405), and three birds (72, 79, and 194) from experiment No. 2, had slight infections of Ascaridia columbae (100 to 400 E.P.G.). Each bird was treated with a piperazine salt administered in the drinking water, and two weeks later with thiabendazole powder mixed with the feed at a dose of 0.5% thiabendazole for a 10-day period, (Wehr and Colglazier, 1968). Fifteen days after the last medication fecal tests were repeated, and helminth eggs were not demonstrated in a 10 day period of examination. Since all pigeons were then negative for Ascaridia, they were considered suitable for these experiments.

2) Source and Culture of Eggs:

Pigeon fecal samples were periodically collected from a nearby farm at Riley, Riley County, Kansas. These were analyzed for helminth eggs using a salt flotation technique. A. columbae eggs were recovered, concentrated, and kept under refrigeration at 4°C until a medium sized inoculum was completed (3,000 to 5,000 unembryonated eggs). Eggs were washed twice in saline and placed in a Petri dish containing 0.1 N H_2SO_4 to a depth of 6-8 mm. Eggs were incubated at room temperature for 30

*Ralston-Purina Company, St. Louis, Mo.

days. At this time eggs were fully embryonated, and two young pigeons were orally infected by crop intubation using a blunt 5 ml. pipette. The infected pigeons were sacrificed by cervical disarticulation 50 days post infection, the small intestine removed, and adult female A. columbae were recovered. These worms were thoroughly washed in tap water, rinsed in 0.85% physiological saline solution (PSS), and placed in a Petri dish containing PSS. This was incubated at 39°C in order to induce oviposition. Two days later, the unembryonated Ascaridia eggs were collected, separated from the female worms and the PSS, and placed in a petri dish containing 0.1N H_2SO_4. They were kept at room temperature for 30 days to obtain large numbers of fully embryonated eggs in the solution.

During incubation the eggs were aerated by gentle agitation daily. These embryonated eggs were used in experiment No. 1, as a source of A. columbae infective larvae (L2) in experiment No. 2, and to carry out studies on factors affecting the levels of oviposition in female worms in experiment No. 3. Ascaridia columbae eggs, like other ascarid eggs easily stuck to the glassware. All glassware was coated in advance with a water soluble silicone compound (Siliclad*) in order to ease egg handling.

3) Experiment No. 1

Ten 10-to 11 week old pigeons, screened for gastrointestinal parasites were randomly separated into five pairs, and orally infected with four different doses of fully embryonated A. columbae eggs (Table 1).

a) Counting and administration of embryonated eggs.

Embryonated eggs were transferred to silicone coated centrifuge

---

*Clay Adams, New York, N.Y.

tubes, and washed in 0.85% physiological saline solution (PSS) three times by centrifugation, at 1500 rpm (350 g)/5 minutes, in order to remove the embryonation medium.

Eggs were concentrated in a 5 ml.-saline volume, thoroughly mixed using a Vortex Genie mixer*, one drop of the suspension was quickly removed using a 1 ml. pipette, placed on a clean slide, and embryonated eggs were counted under the microscope. Following this procedure 4 different doses were established, 250, 500, 1,000 and 2,000 embryonated eggs, and administrated to five pairs of pigeons by crop intubation.

b) Length of infection.

Orally infected pigeons were generally sacrificed by cervical disarticulation 50 days post-infection. Only 1 pair had a 55 days infection.

c) Recovery of larvae and adult worms.

Immediately after pigeons were killed the intestine, liver, lungs, and kidneys were removed. The small and large intestines were separated, slit longitudinally and the contents washed into separate beakers. The gut was rinsed twice with saline to ensure removal of immature stages of A. columbae. Adult worms were transferred to Petri dishes, washed twice with PSS, separated into male and females under a dissecting microscope, and counted. Before storing, the nematodes were dropped in hot glycerin-alcohol, and their length directly measured and recorded. The beaker's sediments were also examined under a dissecting microscope, and larvae were collected, counted and stored. The gut wall of each section

---

*Scientific Products, Evanston, Ill.

was digested overnight in 1% pepsin and 0.5% HCl according to the formula used by Mabon and Reid (1973). The digest was washed through a 80 mesh sieve and left in a refrigerator overnight. The supernatant was removed and the sediment analyzed as described above. The liver, lungs, and kidneys of pigeons 401, 404, 405, and 406, were separately minced using an scalpel, and the Baermann technique was carried out in order to retrieve any migrating A. columbae larvae.

d) Histopathologic techniques.

Tissue samples including liver, lungs, small intestine, spleen, and brain, were removed from pigeons 401, 402, 404, 405, and 406, fixed in 10% formalin, embedded in paraffin, sectioned at approximately 6μ, and stained with Harris's hematoxylin and eosin (H and E).

4) Experiment No. 2.

Twelve 14-18 week old pigeons, screened for gastrointestinal parasites, were randomly divided into six groups, wing banded, and utilized in parenteral infections.

a) Source of embryonated eggs.

Fully embryonated A. columbae eggs from the stock of eggs previously prepared were used. They were washed and pretreated to remove the embryonation medium (0.1N H_2SO_4).

b) Hatching technique.

Infective larvae of A. columbae (L2) or of any other ascarid can not be obtained in large numbers for experimental purposes, except by use of an in vitro hatching technique. Techniques previously described were tried (Hansen et al. 1956; Fairbairn, 1961; and Bindseil 1970). However,

reasonable hatching percentage was not achieved. The method published by Jaskoski and Colucci (1964) and modified by Levine and Silverman (1969) was attempted. This method yielded a high percentage of hatchability.

Fully embryonated Ascaridia columbae eggs were transferred to a centrifuge tube containing a mixture of equal parts (2 ml.) of 1M sodium hydroxide and 5% sodium hypochlorite (Chlorox) for 3.5 to 4 hours at room temperature (25°C). The eggs were then decoated and deshelled. The chlorine and sodium hydroxide were removed, embryonated eggs were washed 5 times in sterile distilled water by centrifugation at 1,500 rpm (350 g)/5 minutes, until no sodium hypochlorite odor remained in the centrifuge tube. All glassware, rubber hoses, stoppers, and solutions were sterile when this technique was performed. Afterwards, 6 ml. of sterile physiological saline solution (PSS) were added to the eggs, and axenic hatching was induced by bubbling CO_2, from an incubator whose atmosphere had 5% CO_2, through a sterile Pasteur pipette tightly fitted to the centrifuge tube stopper (sterile cotton). This pipette had a very fine tip in order to deliver CO_2 bubbles as small as possible through the egg suspension. The CO_2 was delivered at a rate of approximately one cubic foot per hour.

The percent hatchability was monitored every 30 minutes, and the whole technique lasted 1 1/2 to 2 hrs. The centrifuge tube with the embryonated egg suspension was maintained in a water bath at 38°C.

Free hatched larvae were isolated from the unhatched eggs, egg-shell debris, and from dead free larvae by the Baermann technique. Hatched L2 A. columbae (Plate 1, figs. 1 and 2) were allowed to migrate through a flat piece of glass wool 6 to 10 mm. thick which rested on a discshaped base of wire screen. Within 3-6 hours most larvae were in the bottom of the tube in the Baermann apparatus. The time interval between collection of hatched larvae and parenteral injection into pigeons was less than one hour.

The larval suspension was checked for sterility on blood agar plates, which were kept in an incubator at 38°C for 72 hours.

c) Counting and administration of infective L2 A. columbae.

Artificially hatched larvae were counted in the same fashion as done with embryonated eggs in experiment No. 1, except that aseptic technic was observed; the criteria followed for defining a hatched larva was the same as stated by Fairbairn (1961). Hatched larvae were concentrated in a 7 ml. volume of sterile saline containing an inoculum of no less than 600 ± 50 free A. columbae infective larvae per 0.5 ml. of the suspension.

Pigeons were intravenously injected into a wing vein using a tuberculin syringe, and a 25-gauge hypodermic needle. A total of 0.5 ml. of medium plus hatched larvae was injected.

d) Length of infection.

Parenterally infected pigeons were fasted 24 hours before being sacrificed by cervical disarticulation. They were sacrificed according to the following schedule: first pair 8 days post infection (PI) second 16 days PI, third-24 days PI, fourth-32 days PI, fifth-40 days PI, and sixth pair, one at 50 days, the other at 53 days postinfection (Table 5).

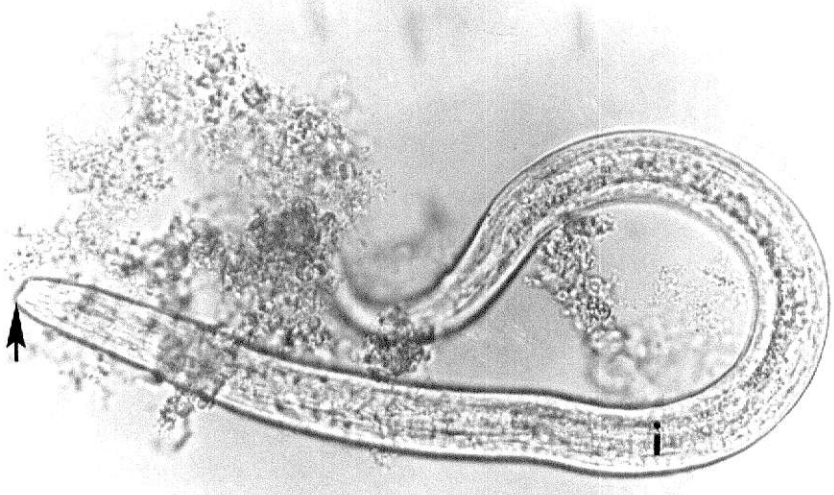
PLATE 1

Figure 1. Second stage or infective larva (L2) of Ascaridia columbae. Penetrating Knob (arrow); i, intestine. X350.

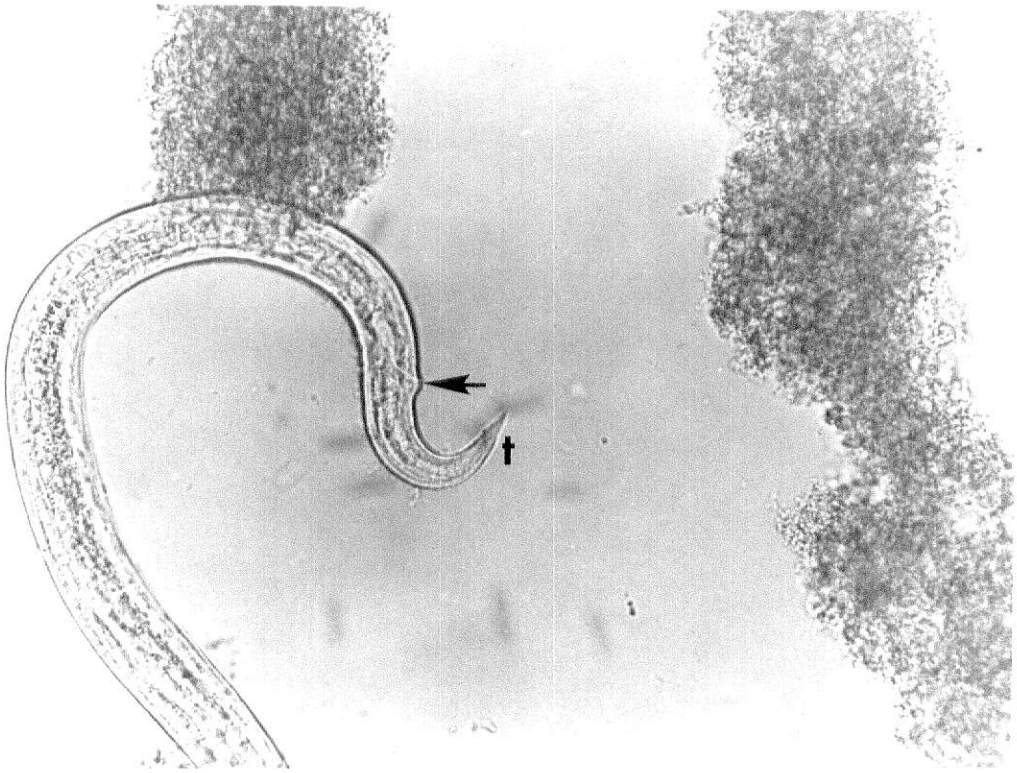
Figure 2. Posterior extremity of L2 A. columbae. Anus (arrow); t, tapering tail. X350.

THIS BOOK CONTAINS NUMEROUS PAGES WITH PICTURES THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.



1



2

e) India ink injections.

Injections of this inert material were administered intravenously to pigeons No. 72, 38, and 37 (Table 5) on the seventh and eighth day post infection. Higgins' India ink* was processed for parenteral injection following the procedures described by Lickley and Monkhouse (1962), and Salvidio and Crosty (1960). India ink was filtered, autoclaved for 10 minutes, and diluted in sterile saline solution at a rate of 3:5. A single dose of 0.5 ml. of diluted India ink was injected intravenously using a tuberculin syringe. Diluted ink was administered slowly at a rate of 0.5 ml. within 2 minutes.

f) Recovery of larvae and adult worms.

Tissues and organs of sacrificed pigeons were immediately examined for A. columbae immature stages and/or adults. The small and large intestines were removed, stripped of mesenteries, and divided into three portions approximately 15 cms. long. They were attached to a faucet and the contents flushed out with warm tap water, (Tugwell and Ackert, 1952). Collected worms were processed following the procedure already explained, and employed for nematodes retrieved from orally infected pigeons. Gut walls were artificially digested using the artificial digest fluid before cited, Maban and Reid (1973, op. cit.,). The Baermann Technique was used to identify wandering and migrating A. columbae larvae in liver, lungs, kidneys, brain, striated muscle (breast), spleen plus heart, and viscera (pancreas, gizzard, and genital organs). The tissues were minced with a sharp knife. The Baermann apparatuses were left at room temperature for 4 hours, the liquid collected analyzed under a dissecting microscope

---

*Faber-Castell Corporation, Newark, N. J.

set at 30 magnifications. Recovered larvae were counted and kept in glycerine-alcohol until morphological studies were completed.

The trachea was examined for parasites by flushing warm tap water through it. Parasites were collected in Petri dishes, and observed under a dissecting microscope.

g) Histopathologic techniques.

Tissues samples from liver, lungs, kidneys, and spleen were routinely taken from sacrificed birds, fixed in 10% formalin, embedded in paraffin, sectioned, and stained with H and E, and periodic acid-Schiff (PAS), and examined microscopically.

Tissue samples from the brain, cerebellum, intestine, thymus, bursa, and muscle were occasionally chosen for microscopic examination.

5) Experiment No. 3.

Some factors affecting the oviposition of female A. columbae in vitro were assessed in this experiment.

a) Origin of nematodes.

Two 10-weeks old pigeons (In Table 1 identified as No. 66 and 50), were orally infected, 3 days apart, with 1,000 embryonated eggs of A. columbae. Infections had a length of 55 days, and bird 50 was sacrificed first by cervical disarticulation. The small intestine was removed, cut lengthwise, and the adult worms present were gathered. Nematodes were separated as to sex and were thoroughly washed in running tap water for 2 minutes, followed by two rinses in distilled water, and three in PSS. These nematodes were used to prepare a preconditioned saline solution containing secretory and excretory products (SEP) of A. columbae.

TABLE 1. TOTAL NUMBER OF *ASCARIDIA COLUMBAE* INFECTIVE EGGS NECESSARY TO PRODUCE 1 WORM IN ORALLY INFECTED PIGEONS.

BIRD NO.	SEX	AGE WKS	DURATION OF INFECTION DAYS	DOSE (NO. EMBRYONATED EGGS)	WORM				NO. EGGS NECESSARY TO PRODUCE 1 WORM
					NO. MALES	NO. FEMALES	TOTAL	MEAN	
406	F	11	50	2,000	153	196	349	600.5	3.3
402	F	10	50	2,000	487	374	852		
401	M	10	50	1,000	234	215	449	324	3.0
403	M	10	50	1,000	96	103	199		
405	M	11	50	500	166	189	355	288	1.7
408	F	11	50	500	93	228	221		
404	F	11	50	250	2	1	3	2.5	100
432	F	11	50	250	1	1	2		
66*	F	10	55	1,000	31	37	68	68.5	14.5
50*	M	10	55	1,000	30	39	69		

*BIRDS INFECTED WITH EMBRYONATED EGGS KEPT IN REFRIGERATOR FOR 4½ MONTHS.

b) Preparation of preconditioned saline media.

Washed adult A. columbae were divided into 3 groups.

Group 1: 20 adult females

Group 2: 20 adult males

Group 3: 10 females and 10 adult males.

Each group was placed in a silicone-coated Petri dish containing 40 ml. of sterile 0.85% saline solution. Petri dishes were introduced into an incubator (38°C) for 48 hours when the saline solutions were removed, and stored at 4°C. Forth ml. of fresh saline were poured into the dishes containing worms, and incubated for an additional 48 hours. This saline was removed, added to the solutions previously collected, and labeled as 48 hours-female solution, 48-hrs male solution, and 48-hrs male-female solution. These solutions were supposedly contained secretory and excretory products (SEP) of the nematodes kept in them. Sterile distilled water was used for preparing the natural and preconditioned saline media.

c) Female A. columbae in normal and preconditioned saline media.

A second group of female A. columbae was collected, from pigeon 66. Worms were gathered and washed as previously explained, and divided into 5 groups having 7 worms per group. Male A. columbae were washed, counted, measured, and kept in storage. In order to assess the effects of SEP of male and/or female A. columbae on nematode egg output groups of 7 female A. columbae, recovered from pigeon 66 were placed in 5 normal and preconditioned media (Table 2). Antibiotics were added to the preconditioned media, and to plain saline to control bacterial growth, avoiding further contamination of the media, and to determine if antibiotics modified the rate of oviposition. Antibiotics added were penicillin G,

5,000 IU/ml. and streptomycin 2.5 mg/ml. of media (Crandall, Echevarria, and Arean, 1963).

Five silicone coated Petri dishes, containing groups of 7 adult female A. columbae and a known medium (Table 2), were incubated at 38°C for 96 hours. Oviposition was induced in the nematodes in all Petri dishes a few hours later. Oviposition was evaluated by counting the total number of A. columbae eggs laid by each group of 7 worms in each dish every 24 hours. Four counts were done. Natural and preconditioned media were substituted by fresh media once 48 hours after the start of the experiment.

d) Technique for counting A. columbae eggs laid in vitro.

- Oviposition Petri dishes were removed from the incubator every 24 hours for egg counts. Female A. columbae were picked up with dissecting needles and maintained in saline at room temperature.

- Media was poured into silicone-coated centrifuge tubes or transferred by a Pasteur pipette.

- Tubes were centrifuged at 2,500 r.p.m. (900 g) for 5 minutes.

- Supernatants consisted of natural or preconditioned saline media. They were reused after the first and third counting, and discarded after the second and fourth.

- Used or new media were poured into new silicone-coated Petri dishes, the female worms dropped into them and reincubated.

- A. columbae eggs were at the bottom of centrifuge tubes, and many remained adhered to the bottom of the Petri dishes. In order to gather and count as many eggs as possible, 20 mls. of 1M NaOH were added to the dishes, and 3-5 ml. to centrifuge tubes. After 4 hrs. eggs were

TABLE 2. SOLUTIONS AND ELEMENTS PRESENT IN NATURAL AND PRECONDITIONED SALINE MEDIA.

MEDIA	VOLUME IN EACH PETRI DISH (ML.)	NO. WORMS	ANTIBIOTICS
MALE SOLUTION	30	7	+
FEMALE SOLUTION	30	7	+
MALE-FEMALE SOLUTION	30	7	+
SALINE PLUS ANTIBIOTICS	30	7	+
SALINE	30	7	-

+ = DRUGS ADDED

decoated, (Ward and Fairbairn 1972, and Copeman and Gaafar 1972), and easier to handle.

- Retrieved eggs from each dish were poured into centrifuge tubes containing their respective sediments plus NaOH. Egg suspensions were thoroughly homogenized using a Vortex Genie mixer for 30 seconds.

- Tubes were centrifuged at 2,500 rpm (900 g) for 5 minutes, the NaOH was discarded, and saline solution was added up to a volume of 5 ml.

- Egg suspensions were again homogenized for 30 second in the mixer. One drop was quickly drawn and placed on a slide using a silicone-coated 1 ml. pipette. Eighteen X18 mm. coverslips were used and eggs were counted under a microscope at 100 X magnifications.

- Three counts were performed every 24 hours for each suspension of eggs plus medium. The results were expressed in total number of eggs laid for 7 adult female A. columbae, during every 24 hour periods. Those results are presented in graph 2.

e) pH of normal and preconditioned saline media.

pH was another factor taken under consideration when carrying out experiment No. 3. The concentration of hydrogen ions for each medium was determined using an electronic pH meter*. The pH of the 2 media were measured before starting the experiment, at 0 hours, 72, and 96 hours.

6) Statistical Analysis.

Data collected from experiments 1 and 2 were statistically analyzed. Body length of nematodes removed from orally and intravenously injected

---

*International Laboratory Inc., Lexington, Mass.

pigeons was the first variable under consideration. A two-way analysis of variance for length was used. In order to determine if the ratio of male to female worms was influenced by the route of infection (oral or parenteral), a chi-square value (X^2) was calculated using contigency tables for sex versus treatment. Data from experiment 3 was also analyzed by the two-way analysis of variance. Statistical analyses were completed in the SAS computer program (SAS Institute Inc., Raleigh, N.C.).

III. RESULTS

1) Experiment No. 1

a) Population of A. columbae recovered from orally infected pigeons.

The oral administration of five fixed doses of embryonated eggs, brought about five different populations of A. columbae, (Table 1). Inoculum fed to birds 66 and 50 were considered as one additional dose, because those eggs were not the same age as the others. Populations of A. columbae recovered from these birds (50 and 66) were lower than from pigeons 401 and 403 infected with similar doses of embryonated eggs which were no more than 2 months old. These differences in population sizes were expected and are in agreement with results for Ascaridia galli infections (Ackert et al., 1947). A direct relationship existed between the means and/or total number of adult nematodes collected from pigeons 406, 402, 401, 403, 405, and 408 and the size of the inoculum. The greater the number of embryonated eggs administered, the larger the number of retrieved worms. However, the inoculum containing 500 embryonated eggs was more efficient in producing adult worms than the inocula with 2,000

and 1,000 eggs. Seventy one and 44.2% of infective eggs, fed to pigeons 405 and 408, became adult A. columbae, whereas lower percentages were obtained for dose 2,000 (17.4 and 42.6%) and 1,000 (44.9 and 19.9%) dose.

Pigeons infected with 250 embryonated eggs yielded unexpected low populations of A. columbae, Tugwell and Ackert (1952) stated: "Egg doses of 100 or 200 were shown by Ackert, Graham, Nolf, and Porter (1931) and later by Ackert, Cooper, and Dewhirst (1947) to be suitable for comparative fowl ascarid studies." Oral infections carried out on pigeons 404 and 432, using a light inoculum of A. columbae infective eggs, did not duplicate the results of Ackert et al., A. galli experiments.

L2 Stages were recovered from the liver of birds number 401, 404, 405, 406, in the amounts of 18, 18, 7, and 92 respectively, whereas none were recovered from other organs.

b) Number of A. columbae embryonated eggs required to produce one worm.

The total number of A. columbae infective eggs, needed to produce 1 worm, in pigeons infected per os with different doses of eggs and with infections having a length of 50 days was calculated. This information was obtained by dividing the size of inoculum by the mean number of worms recovered from each pigeon. Inoculum 500 was most efficient in bringing about adult A. columbae as only 1.7 infective eggs were required to produce a nematode. On the other hand, inoculum 250 had an index of 100 embryonated eggs to yield a single worm.

c) Body length of A. columbae in 50 and 55 day infections.

Nine to 13% A. columbae out of the total nematode populations recovered from birds 66, 401, 402, 403, 404, 405, and 406, were directly

measured, and the data is shown in table 3. The longest adult A. columbae were obtained from pigeon 404, males with an average length of 40 m.m. range 38 to 42 m.m., and one female with a length of 48 m.m. It should be pointed out that only 3 nematodes were collected from this pigeon. The "normal" body length of adult A. columbae has been reported as follows: Males, 29 to 31 m.m., females, 31 to 37 m.m., (Irwing-Smith, 1920, op. cit.), males 16 to 31 m.m., females 20 to 40 m.m., (Mozgovoi, 1953, op. cit.), and males, 22.2 m.m., females 25.9 m.m., (Wehr and Hwang, 1964, op. cit.). The length of almost all the measured worms obtained from other pigeons, fell within normal limits.

d) Macroscopic and Microscopic observations.

Gross appearance of internal organs. Macroscopic lesions were observed in the small intestine and the liver. The intestinal lumen was dilated, the walls were thin and rather transparent, and A. columbae were visible through them. Numerous 1-2 mm size lesions were seen on the surface of the liver of orally infected pigeons, especially in those which received doses of 1,000 or more embryonated eggs. The genesis and development of those lesions may be similar to the ones caused by Ascaris suum L2 stage in liver of pigs, commonly named "hepatic" white spots, (Roneus, 1966).

Microscopic observations. The microscopic lesions in the liver of orally infected pigeons, caused by migrating L2 A. columbae, has been reported by Wehr and Shalkop (1963, op. cit.). Tissues from pigeons infected with 2,000 embryonated eggs, had multiple granulomatous lesions (Plate 2, figs. 3, 4, and 5) characterized by the presence of foreign-body

TABLE 3. LENGTH OF ASCARIDIA COLUMBAE FROM ORALLY INFECTED PIGEONS.

BIRD NO. AND SEX	DURATION OF INFECTION (DAYS)	DOSE (NO. EMBRYONATED EGGS)	MALES		FEMALES	
			NO. WORMS MEASURED	$\bar{X}$ (RANGE IN M.M.)	NO. WORMS MEASURED	X (RANGE IN M.M.)
402 - F	50	2,000	50	27 (22-31)	50	35 (30-42)
406 - F			40	29 (26-30)	40	40 (35-44)
401 - F	50	1,000	40	29 (24-35)	40	36 (30-43)
403 - M			25	28 (25-31)	25	37 (32-43)
66 - F*	55	1,000	20	32 (29-35)	20	39 (29-44)
405 - M	50	500	40	27 (22-31)	40	35 (31-40)
404 - F	50	250	2	40 (38-42)	1	48 ---

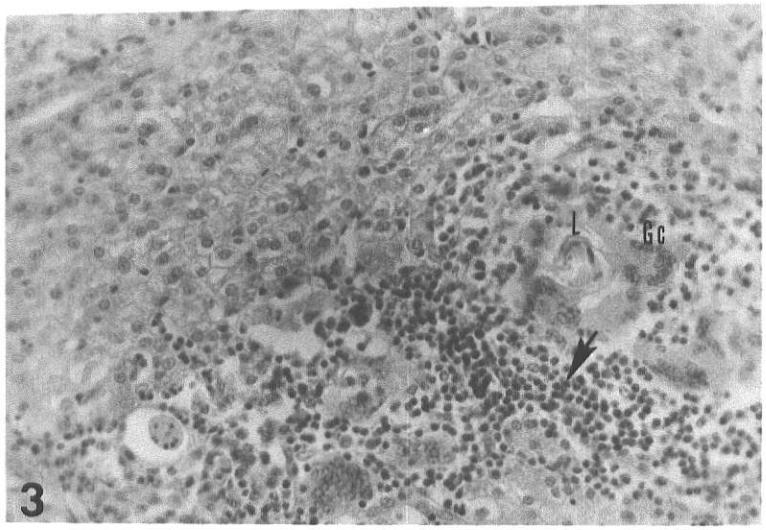
*BIRDS INFECTED WITH EMBRYONATED EGGS KEPT IN REFRIGERATOR FOR 4½ MONTHS.

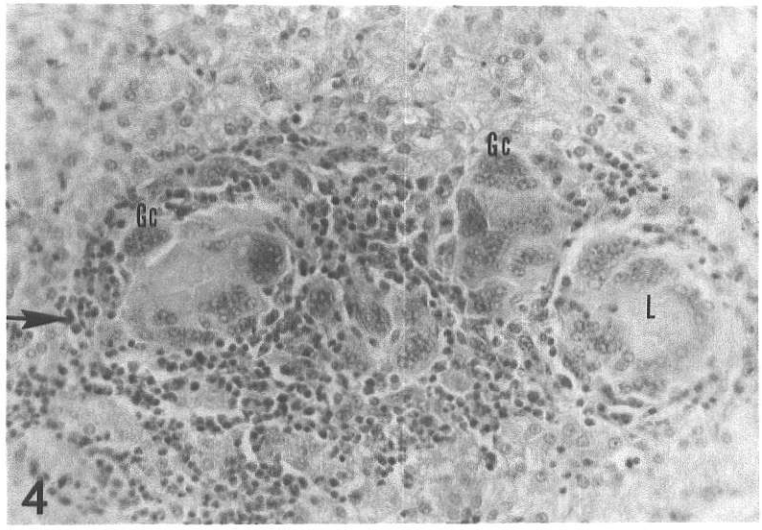
PLATE 2

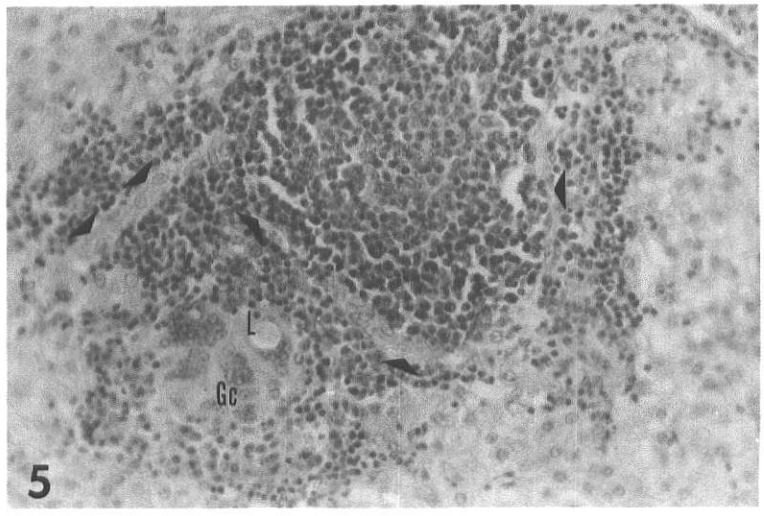
Figure 3. Liver section from orally infected pigeon showing granulomatous lesions. Note groups of giant cells (Gc) clustered around fragment of A. columbae larva (L). Lymphocytes (arrow) are also involved in the inflammatory reaction. X350.

Figure 4. Liver section from orally infected pigeon showing a group of multiple granuloma. Giant cells (Gc) of foreign-body type and mononuclear cells (arrow) surround fragments of A. columbae larvae (L). X350.

Figure 5. Liver section from orally infected pigeon showing germinal centre-like lesion limited by a layer of connective tissue (arrows). A group of giant cells (Gc) enclosing larval fragments (L) is also observed. X350.


L
Gc
3


Gc
Gc
L
4


L
Gc
5

type, multinucleated giant cells often clustered around fragments of A. columbae larvae (Figs. 3 and 5). Eosinophils and mononuclear cells were interspersed or around the giant cells. Larvae were surrounded by cells characteristics of a cellular immune reaction, and no intact larvae were seen in the slides studied.

2) Experiment No. 2

a) Efficacy of hatching techniques.

Four hatching techniques were compared and the results are presented in table 4. Percentages of hatching were calculated after counting a total of 200 to 300 free hatched larvae of A. columbae plus unhatched eggs in a suitable diluted drop of medium. Percentages of hatched larvae were inferred from the total figures counted and above cited.

b) Distribution and stage of development of A. columbae in intravenously infected pigeons.

The distribution, location and number of worms retrieved from parenterally infected pigeons is presented in table 5. Second larvae and L3 A. columbae were gathered from lung tissues of pigeons 72, 79, 194, 192, and 45. Second larval stages of A. columbae retrieved from pigeon 72 (8 days postinfection) often showed slow or no movements, had some wrinkles in their cuticle, and many white blood cells (WBC) adhered to the cuticle (Plate 3, figs. 6, 7, and 8), larvae obtained from other birds were generally L3, with high motility. The features used to identify these larvae as L3 stage were their body length (more than 0.500 mm), and the presence of weakly developed lips around their oral cavity (Wehr and Hwang, 1964), (Plate 4, figs 9 and 10).

TABLE 4. EFFICACY OF HATCHING TECHNIQUES TESTED WITH *A. COLUMBAE* EMBRYONATED EGGS.

TECHNIQUE(*)	APPROXIMATE TOTAL NO. OF EGGS USED	NO OF EMBRYONATED EGGS AND HATCHED LARVAE COUNTED	% HATCHING
CASAROSA (IN BINDSEIL, 1970A)	2,000	200	12%
	2,000	300	14%
HANSEN *ET AL.* (1956)	1,000	200	1%
	1,000	200	3%
FAIRBAIRN (1961)	3,900	200	28%
	3,900	300	61%
MODIFIED METHOD OF JASKOWSKI AND COLUCCI (1964)	3,000	200	72%+
	3,000	300	97%++

(*) = 2 TRIALS WERE PERFORMED FOR EACH TECHNIQUE.

\+ = % OBTAINED AFTER 1 HOURS OF HATCHING.

++ = % OBTAINED AFTER 2 HOURS OF HATCHING.

Table 5. LOCATION AND NUMBER OF WORMS RECOVERED FROM TISSUES AND ORGANS OF PIGEONS INTRAVENOUSLY INFECTED WITH 600 ± 50 L_2 STAGE ASCARIDIA COLUMBAE

NO. OF BIRDS AND SEX	DURATION OF INFECTION (DAYS)	NUMBER OF LARVAE RECOVERED (1)								LARVAE AND ADULT WORMS FROM SMALL INTESTINE	
		LIVER	LUNGS	TRACHEA	KIDNEYS	SPLEEN-HEART	BRAIN	STRIATED MUSCLES	VISCERA (2)	INGESTA (3)	GUT WALL (4)
72 M	8	---	74	---	---	1	0	---	---	---	---
79 F	8	---	169	3	---	---	0	---	---	---	---
194 M	16	---	5	---	3	---	---	---	---	---	---
192 F	16	---	21	4	---	---	---	---	---	---	---
45 M	24	---	12	---	---	---	---	---	---	---	---
197 F	24	---	---	---	---	---	---	---	---	---	---
221 M	32	---	---	---	---	---	---	---	---	6	2
24 F	32	---	---	---	---	---	---	---	---	4	2
38 M	40	---	---	---	---	---	---	---	---	5	---
59 F	40	---	---	---	---	---	---	---	---	3	---
22 F	53	---	---	---	---	---	0	0	---	25	---
37 F	50	---	---	---	---	---	0	0	---	27	---

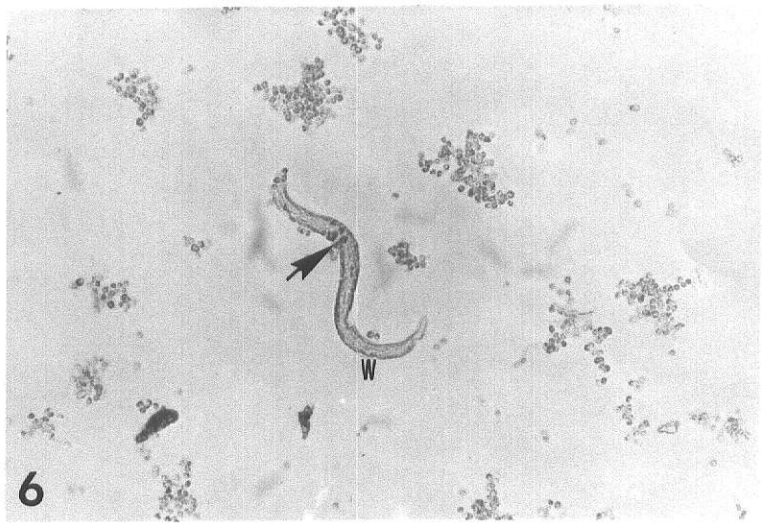
(1) Retrieval of larvae was carried out by the Baerman technique
(2) It included a) pancreas, gizzard, and genital organs, and b) large intestine; a and b were analyzed separately.
(3) Intestines were hooked to a faucet and its contents were flushed out with warm tap water.
(4) Digested overnight in 1% pepsin and 0.5% HCl according to MABON, T. L., and REID, W. M. (1973)

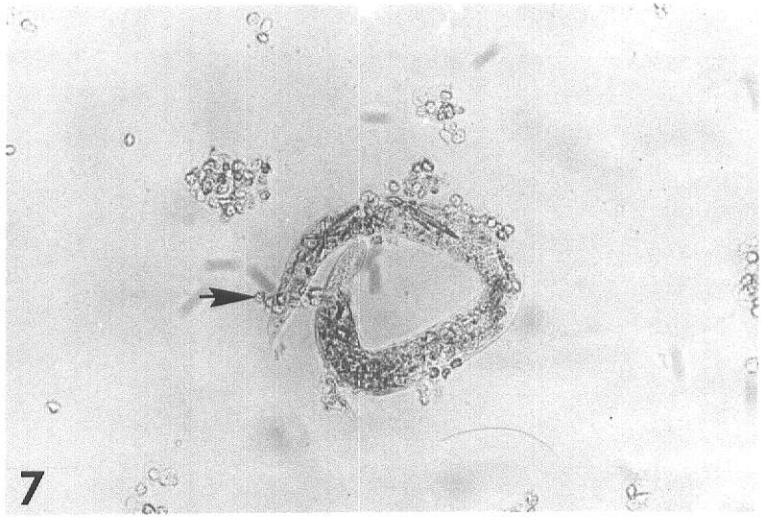
--- = Negative; O = Not examined

PLATE 3

Figure 6. L2 A. columbae retrieved from lungs of pigeon 72 which had a pulmonary fungal disease. Some polymorphonuclear leucocytes (arrow) are adhered to the larva cuticle. This also shows wrinkles (w). X350.

Figures 7 and 8. Another L2 A. columbae obtained from the lungs of pigeon 72. Some inflammatory cells look like eosinophils (arrows). Fig. 7. X350. Fig. 8. X700.


W
6


7

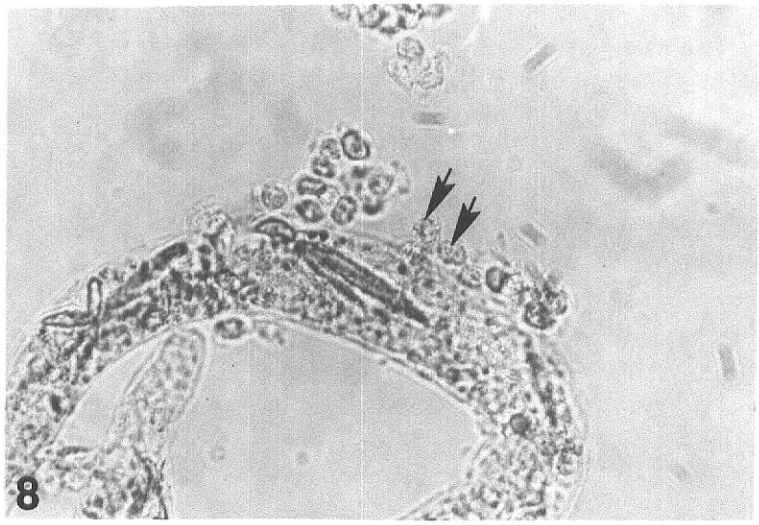
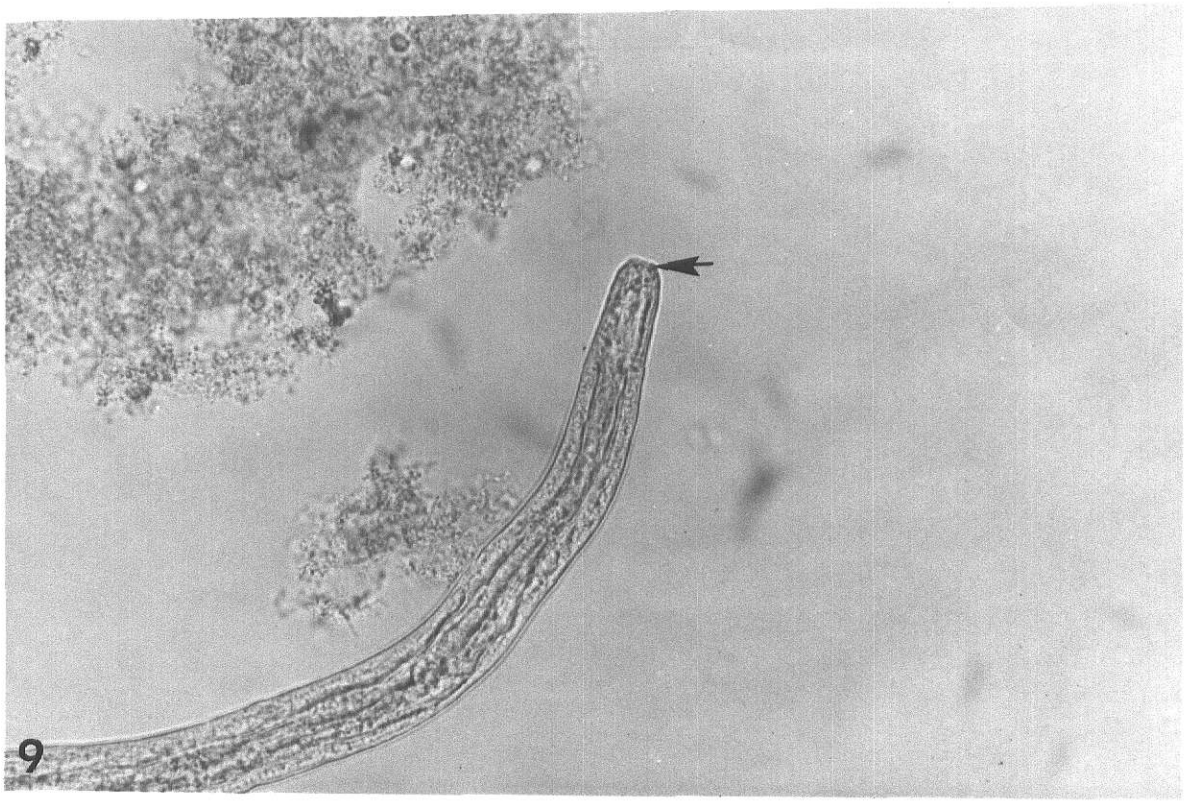
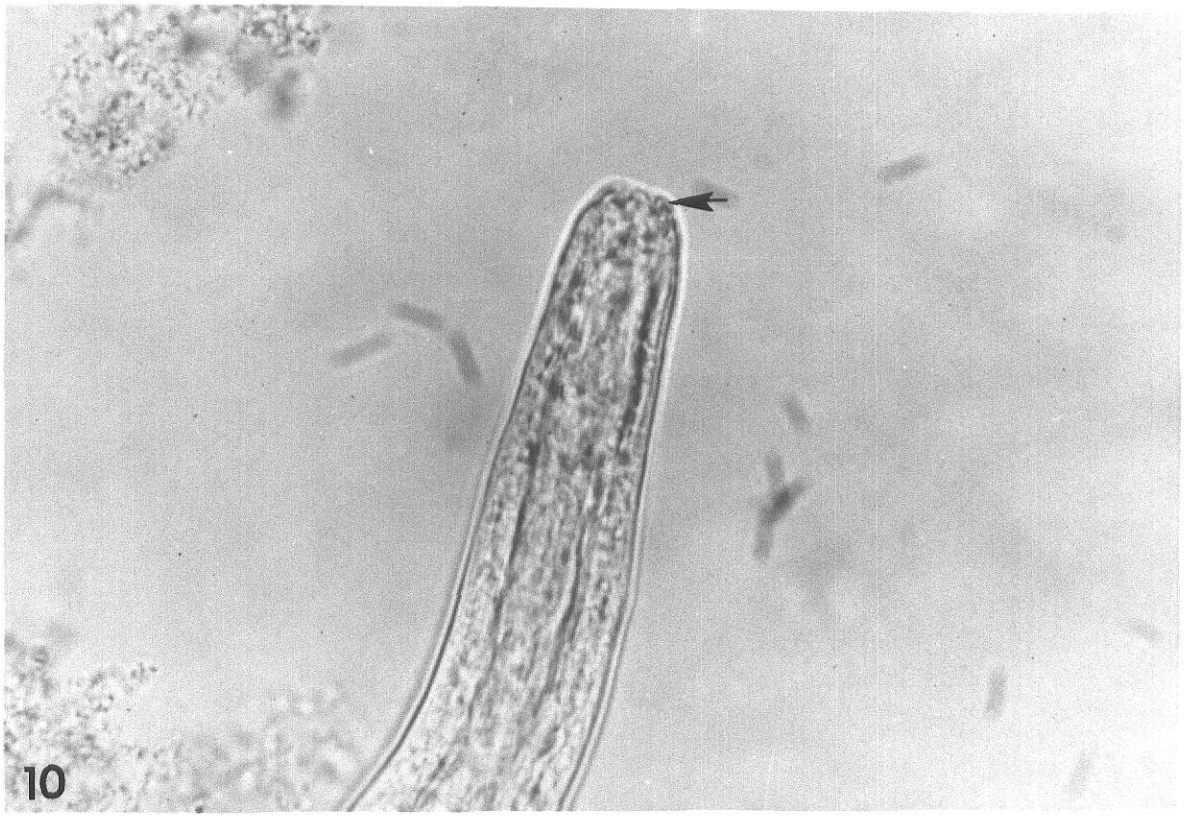

8

PLATE 4

Figure 9. Third stage larva (L3) of Ascaridia columbae collected from lungs of intravenously infected pigeons. Lips (arrow) are distinct, but weakly developed. X350.

Figure 10. L3 A. columbae collected from lungs of intravenously infected pigeon at higher mangification. Rudimentary lips (arrow) are helpful structures identifying this larval stage.


9


10

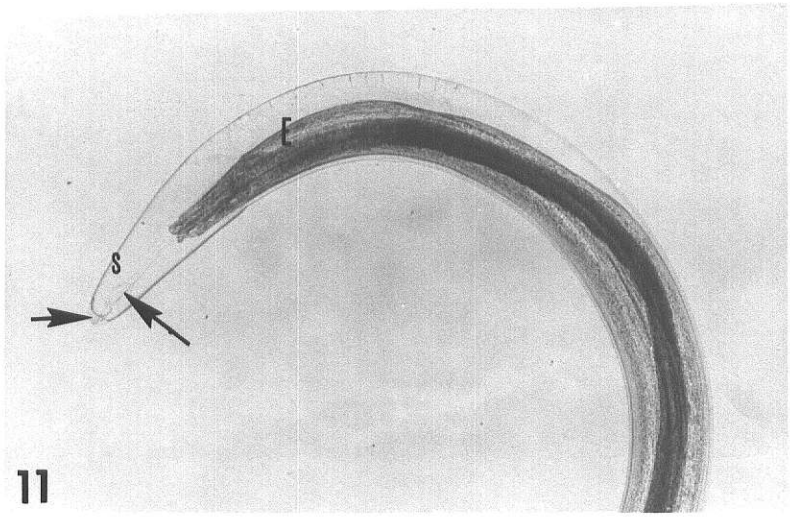
A. columbae larvae were constantly found in lungs of parenterally infected pigeons even 24 days PI. At that time, no larvae were retrieved from liver, brain, striated muscles, viscera, or small intestine. Three larvae were recovered from kidney, one from a spleen-heart mix, and 7 from the trachea. Larvae were concentrated in the respiratory tree of infected pigeons 24 days postinfection. However, from day 32 on, L4, young adult, and adult A. columbae were constantly found in the lumen and/or mucosa of the small intestine. Larvae were never retrieved from liver, brain, striated muscle, or viscera of experimental birds.

Only four L4 A. columbae were found in the gut wall, and they were in the process of molting, but they had not yet shed their skins L4 (Plate 5, figs. 11, 12, 13). The change from L3 to L4 occurred between the 24th and 32th day after infection. Fourth larval stages were identified on the basis of their morphological features. Females had an average body length of 3.5 m.m., and a width of 125μ (taken at the middle of the body). The esophagus was 550μ long, and club shaped. The nerve ring was situated 235μ behind the bucal cavity. The lips were easily seen, and the vulva (Plate 6, Fig. 15) was found in the middle of the body. The anus was 225μ cephalic to the tip of the tail. Fourth larvae, young, and adult females, recovered from the intestinal lumen, constantly showed the characteristic "orbicular corpuscles" (Plate 6, Fig. 14) first mentioned by Dujardin (1845) as cited by Irwin-Smith (1920), but seldom reported by other authors. These corpuscles were round, easily seen in the posterior extremity, smaller in diameter (2 x 2μ) in L4 females, and larger in adult worms (3.5 x 4μ). The nature and possible functions of these "orbicular corpuscles" is unknown, and they are not found in male worms at any evolutive stage.

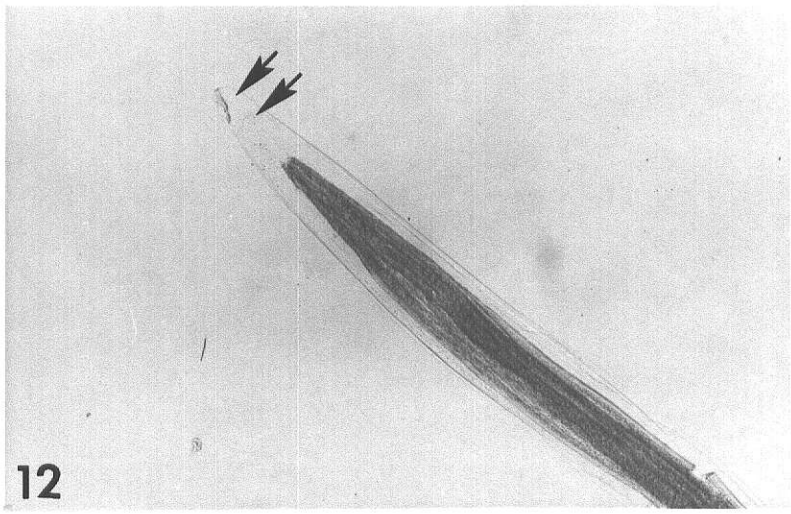
PLATE 5

Figure 11. Anterior extremity of fourth-stage larva of A. columbae obtained from the gut wall of intravenously infected pigeon. Note the unshed larval sheath (S) which shows the impression left by the lips (arrow) and the cervical ala (longest arrow). E, esophagus; i, intestine. X350.

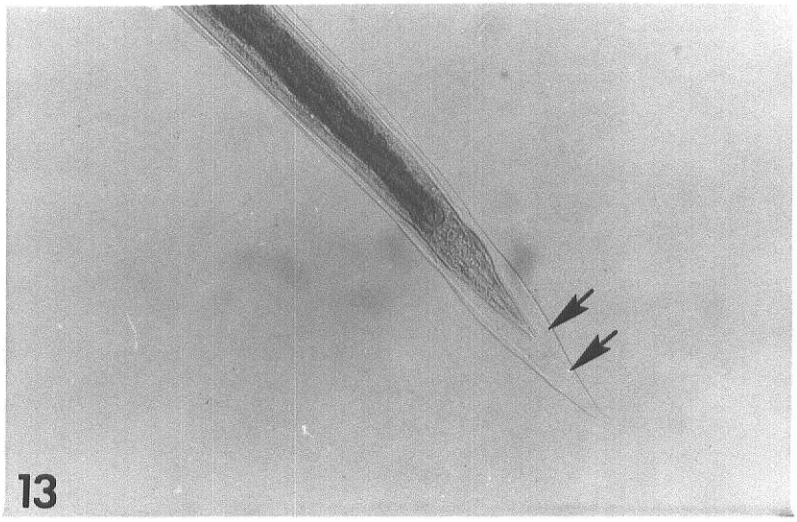
Figures 12 and 13. Anterior and posterior ends of a same L4 A. columbae collected from the gut wall of intravenously infected pigeon. Two sheaths are seen on the same larva (arrows).



11



12



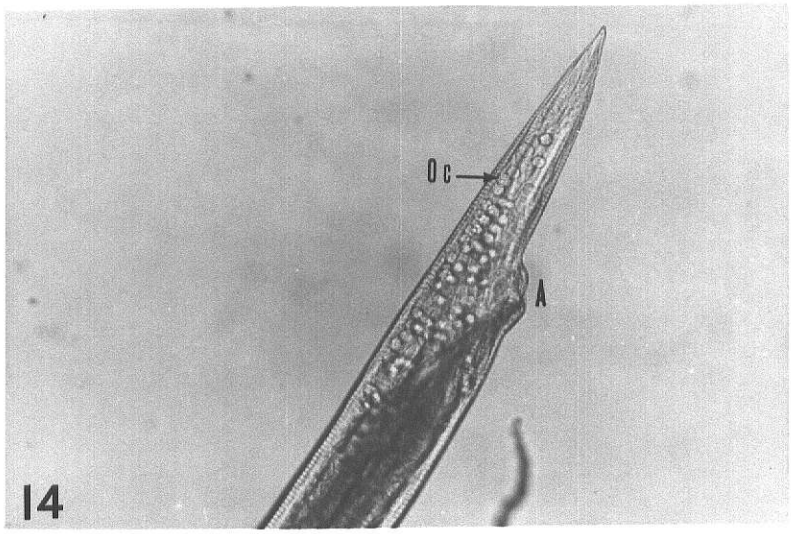
13

PLATE 6

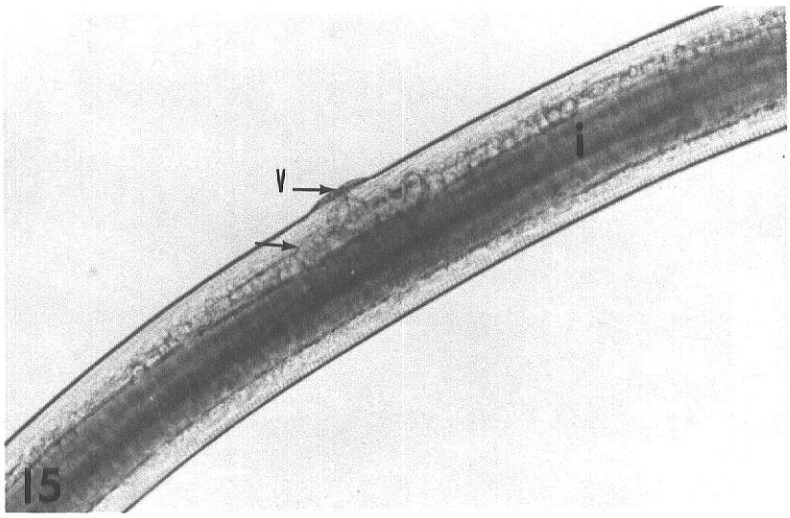
Figure 14. Posterior extremity of young female Ascaridia columbae. O.c., orbicular corpuscles; A, anus.

Figure 15. Vulvar region of young female A. columbae. V, vulva; rudimentary reproductive system (arrow); i, intestine.

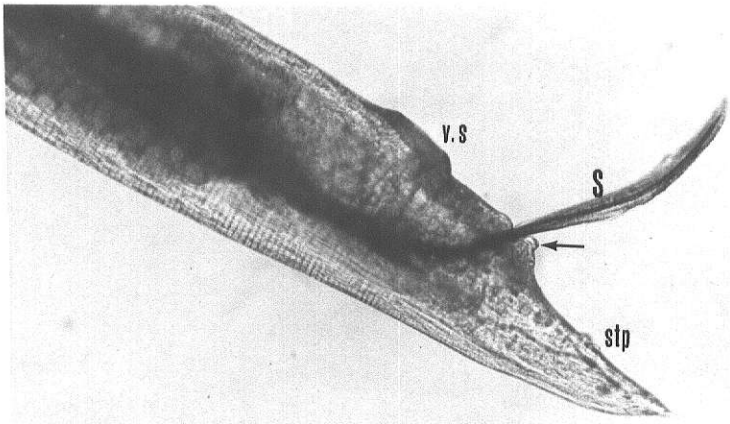
Figure 16. Posterior extremity of young male A. columbae. S, spicules; v.s., ventral sucker; and papilla (arrow); s.t.p., sub-terminal papillae.



14



15



16

A young male recovered from the ingesta of pigeon 24, had a length of 20 m.m., was 475μ wide at the middle of the body, had distinct pre-anal sucker, caudal papillae, and a pair of equal spicules 685μ long (Plate 6, fig. 16).

c) Length of adult A. columbae and size of infections in intravenously infected pigeons.

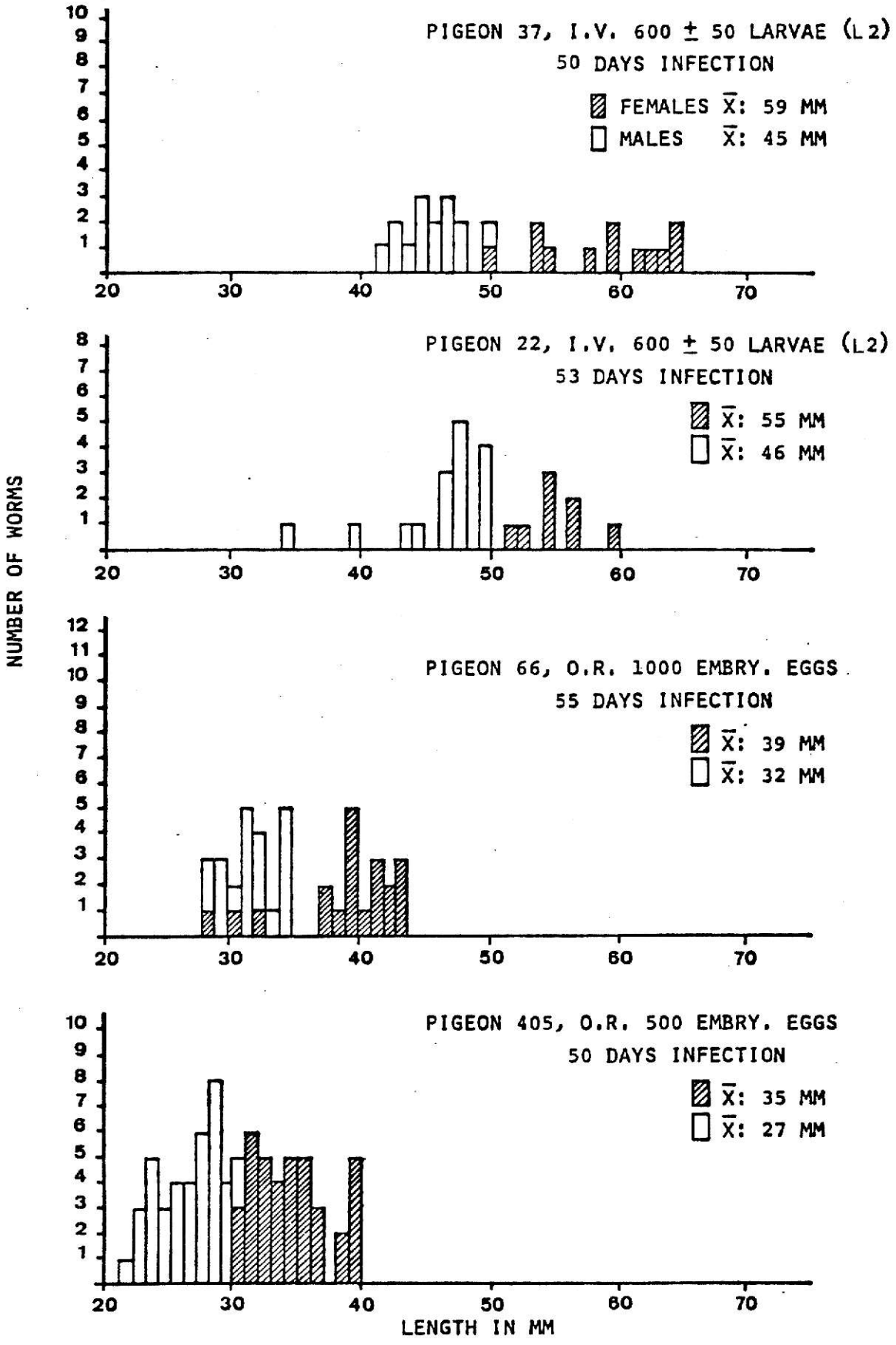
The length of A. columbae immature stages from lungs, intestinal mucosa, and gut lumen, were within the ranges considered as normal for this nematode. On the contrary, adult A. columbae found in the intestinal lumen of pigeons 22 and 37, whose infections lasted 53 and 50 days respectively, were longer than normal. Twelve adult females from bird 37 had an average length of 59 m.m., (range 50 - 65 m.m.), and 15 males obtained from the same bird had an average length of 45 m.m. (range 42-50 m.m.), graph 1. These exceeded the length already mentioned and considered as "normal" for adult A. columbae. Worms recovered from intravenously infected pigeons (22 and 37) were significantly longer ($P < .0001$) than those recovered from the orally infected birds, with the same duration of infection. The chi-square test showed that types of infection, intravenous versus oral, did not significantly ($P > .05$) affect the sex ratio of the worms recovered. The former group (I.V. infected) had not only longer worms, but they also were more robust, with greater diameter than those in the oral infections.

d) India ink injections.

Single intravenous injections of diluted India ink, in pigeons 72, 38, and 37, on the 7th and 8th postinfection days respectively, had no influence on the total nematode counts when compared to the untreated bird

Graph 1. Frequency distribution of the length of Ascaridia columbae from intravenously (I.V.) and orally (O.R.) infected pigeons.

FREQUENCY DISTRIBUTION OF THE LENGTH OF ASCARIDIA COLUMBAE
FROM INTRAVENOUSLY (I.V.) AND ORALLY (O.R.) INFECTED PIGEONS



in the pair. Larval and/or adult worm counts were similar in pigeons 38 (treated) and 59 (nontreated), and between birds 37 (treated) and 22 (nontreated). Differences in larval counts were apparently significant between pigeons 72 (treated) and 79 (nontreated) (Table 5). Reasons for these similarities and differences will be discussed.

e) Post mortem examination. Macroscopic and microscopic observations.

Gross appearance of internal organs. Thoracic and abdominal organs had no macroscopic lesions or degeneration, except for the spleen in birds 79, 192, and 194, which were darker and enlarger than normal.

Microscopic observations. Microscopic changes were generally found in the pulmonary tissues, and few inflammatory reactions were observed in other organs. Eight days after intravenous infection with A. columbae larvae, active lymphoid follicles and focal inflammatory reactions were present in the lungs. These reactions were characterized by the presence of lymphocytes, macrophages, large numbers of eosinophils, and perivascular cuffing of the arterioles. Liver and other organs were normal. Sixteen days post infection microscopic lesions were seen in lungs, and the inflammatory reaction was similar to the one observed 8 days after parenteral infection. A focal concentration of either eosinophils or heterophils was detected in the kidney cortex.

Pulmonary tissues from pigeons sacrificed 24 days post infection (45 and 197) had a marked cellular inflammatory reaction. Fragments of tissues, suspected of being destroyed A. columbae larvae, were surrounded by foreign-body type giant cells. Large numbers of eosinophils, lymphocytes, and macrophages, were also observed in the vicinity of these focal lesions. These lesions were organized granulomas, present in the lung parenchyma,

and were accompanied by slight hemorrhages in some bronchi. Hemorrhages were seen in air passages and septae together with the cellular infiltrations. Other relevant histopathologic lesions in the pigeons above cited, were eosinophilic or heterophilic infiltration in the intestinal mucosa, and reduction or depletion of lymphocytes in the spleen. In this pair of birds (45 and 197) microscopic studies were extended to liver, brain, cerebellum, bursa, thymus, and kidney. No lesions, or A. columbae immature stages were observed, and tissues were normal. Eosinophils and mononuclear cell infiltration were the dominant findings in lung of pigeons sacrificed 32 days after intravenous infection. Granuloma formation accompanied by giant cells were observed in lungs of birds killed 40 days post infection. These lesions were similar to the granulomas already described. In these birds granulomas were not confined to the lungs, but a pair of them were seen in liver. This is hard to explain, as no larvae were retrieved from hepatic tissues by the Baerman technique, and liver tissues from all the other birds did not show any inflammatory reaction. Tissue sections of lungs from birds sacrificed 50 and 53 days post infection, had scattered lymphonodular lesions, a few eosinophils, and there was evidence of healing.

3) Experiment No. 3.

a) Partial and total egg outputs of female A. columbae kept in natural and preconditioned saline media.

Partial and total counts of A. columbae eggs, laid in vitro in natural and preconditioned saline media, are showed in graph 2. Data obtained were broken down into three items.

Graph 2. Partial and total egg outputs of female Ascaridia columbae kept in natural and preconditioned saline media.

The number of eggs laid *in vitro*, every day, in each medium is presented in graph 2. After the first 24 hrs. of oviposition, female *A. columbae* in saline, saline plus antibiotics, and "male-female" solution, laid similar number of eggs with values ranging from 27,500 to 29,500. Eggs counted in the "female", and "male" solution were below 20,000. Only 12,400 were recovered from the "male" solution. Twenty four hours and 48 hours after the experiment began, the total number of eggs in each medium decreased, with counted eggs in saline solution being the largest figure recorded (26,700), and those present in "male" solution being again the lowest number (11,100). *A. columbae* eggs laid 72 and 96 hours after the experiment began, were very low (below 7,500 eggs), and without statistical significance.

A comparison between number of eggs laid *in vitro* in the first 48 hrs of the experiment, and those laid in the last 48 hrs is also presented in graph 2. More than 84% of the counted eggs were recovered from the first 48 hours. The number of eggs laid in the "male" solution during the first 48 hours, represented the lowest figure calculated for any medium. Total oviposition after 96 hrs. is shown at the bottom of graph 2. It should be noted that a slight increase in the partial egg outputs in "female" solution during the last 48 hours of the experiment, contributed to augment the total amount of eggs laid in "female" solution. The number of eggs in the female solution were similar to those obtained from the medium saline plus antibiotics.

b) pH readings.

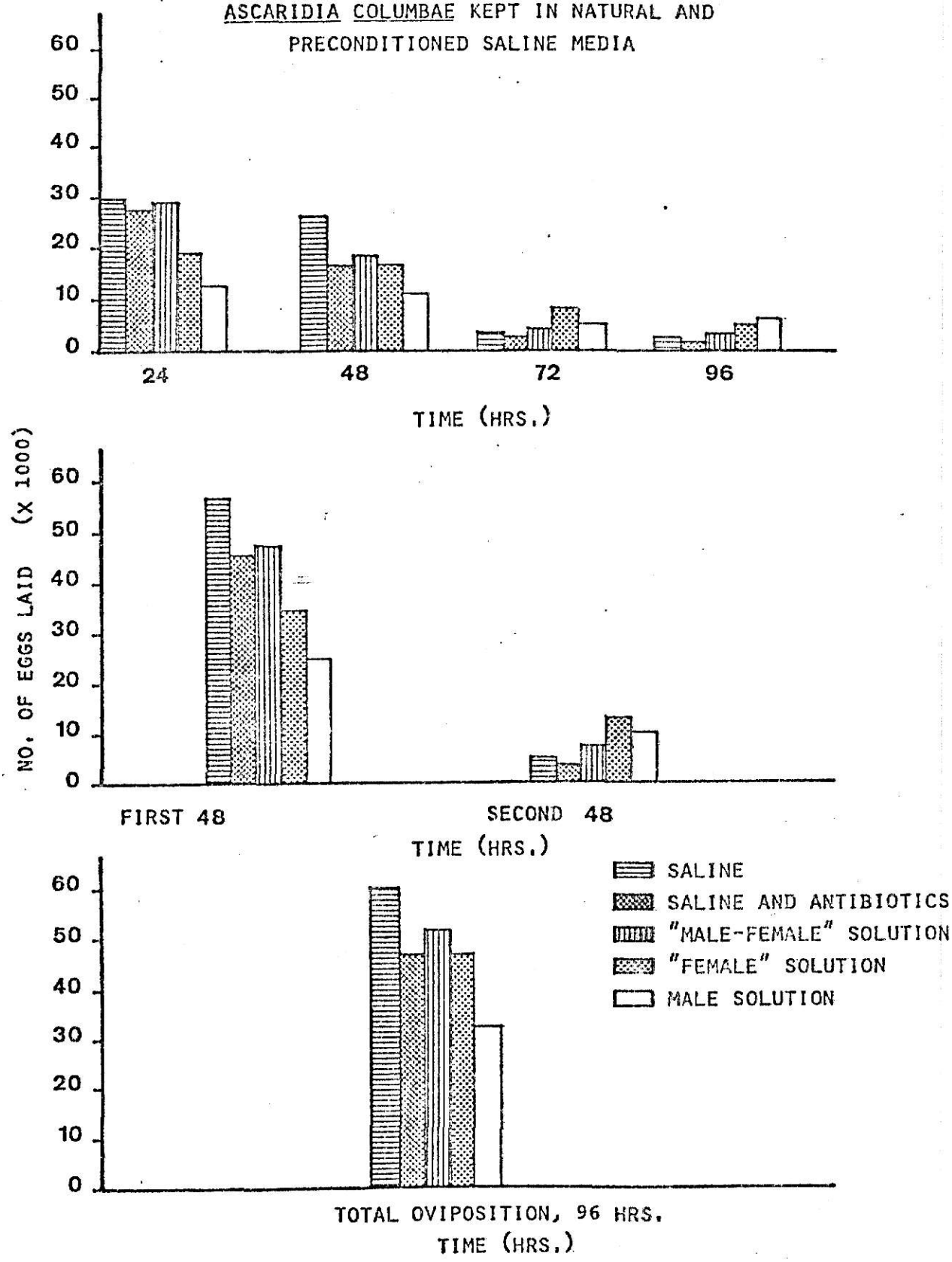
The concentration of hydrogen ions for each medium was read, although the sequence used was not the one wished. Results are showed in Table 6.

TABLE 6. pH VALUES RECORDED FOR THE DIFFERENT MEDIA AT SEVERAL INTERVALS OF TIME.

	FREQUENCY READINGS		
MEDIA	0 HOURS	72 HOURS	96 HOURS
"MALE" SOLUTION	5.5	4.9	4.9
"FEMALE" SOLUTION	5.4	4.8	4.9
"MALE-FEMALE" SOLUTION	5.7	4.9	7.0
SALINE-ANTIBIOTICS	6.1	6.2	6.1
SALINE	6.5	6.0	7.2

Graph 1.

PARTIAL AND TOTAL EGG OUTPUTS OF FEMALES ASCARIDIA COLUMBAE KEPT IN NATURAL AND PRECONDITIONED SALINE MEDIA



Before starting the experiment, media had pH's over 5.4 ("female" solution) and below 6.5 (saline). Seventy-two hours later pHs in the "male" and "female" solution had decreased slightly. A relationship between the partial and total egg outputs in "male" and "female" solutions and their low pH cannot be inferred. Furthermore, total egg outputs were greater in the "female" solution than in the "male" (graph 2), and "female" solution had a more acid medium than "male" solution. Normal values of pH for squab and pigeon small intestines are within 5.23-5.89 (Crompton and Nehseim, 1976), which indicates that A. columbae oviposition is naturally carried out in acid media.

IV. DISCUSSION

Many aspects on the biology of domestic fowl nematodes were covered in the past decades especially by Dr. Ackert and his coworkers at Kansas State University. The scope of those studies ranged from investigations on the morphology and life history of fowl nematodes (Ackert, 1917, 1931), to mechanisms of resistance and immunity to parasitism (Frick and Ackert, 1948). The biologic system Ascaridia galli in chickens was invariably used in their experiments. Nowadays, it still has priority on other biologic systems when fowl nematode parasitism is ascertained, either in studies assessing immunosuppressive drugs in fowl roundworms (Johnson, Hansen and Nassar, 1974), or in the case of research on arrested development in avian species (Ikeme, 1970).

Other biologic systems, i.e. Ascaridia columbae-pigeons, Ascaridia dissimilis-turkeys, and Ascaridia numidae-guinea fowls, have occasionally

been considered by researchers when planning studies on nematode parasites of domestic fowl. The system A. columbae-in pigeons, was chosen when conducting this research in order to ascertain some aspects still unclear on the life cycle of this helminth in orally and intravenously infected pigeons.

To begin with, one might ask what would be the lowest single dose of embryonated A. columbae eggs, able to cause an infection suitable for comparative pigeon ascarid studies. This figure was worked out for A. galli by Ackert et al. (1947), and it happens to be 100 to 200 infected eggs; however, it is unknown for A. columbae. In oral infections carried out in this research, the dose 500 yielded the best results as far as efficacy in producing adult worms is concerned (Table 1), whereas a dose of 250 infective eggs hardly caused infection in two pigeons. Further trials should be done in pigeons using either 250 or lower doses, 50, 100, or 200 A. columbae embryonated eggs, since it seems surprising that 250 infective eggs did not bring about at least a 5% size infection, just as it occurs in arount 50% of chickens orally infected with 100 $\pm$ 10 A. galli infective eggs (Johnson, Hansen and Nassar, 1974 op. cit.).

One fully embryonated egg of an ascarid or any other nematode, is genetically endowed to generate one worm in its appropriate host; however, natural or experimental infections with one embryonated nematode egg are too hard to accomplish, due to shortcomings like the method of infection, the viability of the larva, the age and breed of the host, the lack of information about early primary infections in the host with

the same parasite, and so forth. A threshold inoculum ought to exist for infecting, i.e. pigeons or chickens with embryonated eggs, above which, in the right conditions, infection is always caused, and below which no adult worms are produced. That threshold inoculum is unknown for Ascaridia columbae and for other helminths of domestic animals.

Regardless of the size of the inoculum, L2 A. columbae were commonly retrieved from liver of orally infected birds, even when using the dose 250. Wehr and Hwang (1959) reported the presence of L2 in extraintestinal tissues (liver and lungs), but they constantly used large doses of infective eggs (5,000). These large doses might have temporarily overwhelmed the mechanisms of the immune response allowing L2 A. columbae to arrive in organs beyond liver, i.e. lungs.

Invasion of the intestinal mucosa by ascarid larvae, their histotropic phase, and migration through extraintestinal organs have been topics of enormous controversy, because the answers to explain such phenomena are contradictory. As far as the genus Ascaridia is concerned, Ackert (1931), and Tugwell and Ackert (1952), concluded that the invasion of the intestinal mucosa by A. galli larvae was a normal and obligatory phase in the development of this nematode. On the other hand, Hansen, Oonyawongse, and Ackert (1952), Horton-Smith, and Long (1956), and Moran and Mizelle (1956), reported that only some L2 stages invaded or were in close relationship with the intestinal mucosa. Lately, Herd and McNaught (1975), studying the histotropic phase of A. galli in chickens, receiving injections of alkylating immunosuppressive agents (Cyclophosphamide), concluded that the invasion of the mucosa and the histotropic phase,

appeared to be a normal part of A. galli life cycle, regardless of the size of the inoculum.

With regard to A. columbae, little information is available. It should be mentioned that Wehr and Hwang (1964) found larvae of A. columbae in both the intestinal scrapings and the washings of sacrificed pigeons; thus, they stated: "A tissue-invading cycle is not essential for the complete development of this species of nematode."

In this research few or no larvae were retrieved from the gut lumen, and/or the intestinal mucosa of orally infected pigeons 50 or 55 days post infection. A total of four L4 A. columbae were collected from the gut wall of intravenously infected birds 32 days post infection (Table 5). Although this data does not have numerous replications it appeared that only a few larvae invaded the intestinal mucosa. This finding is in agreement with Wehr and Hwang (1964).

A look at the extraintestinal migration by Ascaridia sp. immature stages, reveals that larval migration outside of the intestine is carried out at different degrees by different species. Ascaridia numidae does not migrate at all (Mabon and Reid, 1973), A. dissimilis rarely migrates to the visceral organs of turkeys (Horton-Smith, Long, and Lee, 1968), few A. galli larvae have been found in extraintestinal tissues of chickens (Ackert, 1931); and Ascaridia columbae larvae, often migrate from the alimentary canal toward the liver or lungs in pigeons (Hwang and Wehr, 1958, 1959). This last finding led them to suspect that A. columbae life cycle was similar to that of Ascaris suum, and to Michel (1974) to write: "A. columbae normally, and perhaps invariably, performs a tracheal migration."

This contradicted Wehr and Hwang (1964) who had already reported that, (a) its life cycle was direct, and (b) that some larvae, for unknown reasons, only migrated toward the liver of infected birds, and sometimes to the lungs of pigeons infected with multiple doses of embryonated eggs. From an evolutive point of view, it may be inferred that members of the genus Ascaridia found in domestic fowl, are unevenly tolerated by their respective hosts. A. numidae appears to be the best adapted as far as host-parasite interactions is concerned, whereas A. columbae is in the fourth and last place. Applying Sprent's evolutive theories on host-parasite relationships (1959), it can be speculated that in orally infected pigeons some L2 A. columbae have to migrate, in greater or lower degree after arriving in the small intestine, as uneven levels of tolerance exist between A. columbae and pigeons. These differences in adaptation seem to be part of the stimuli which turn on the host's defense mechanisms, in other words, the cellular and/or humoral immune responses. Anti-parasite immunity generated in that way might act from causing inhibition of larval establishment at intestinal levels, to decreasing the growth rate and number of worms in orally infected pigeons. A local immunity mediated by reaginic antibodies (Ogilvie and Jones, 1971; Johansson et al. 1968), lymphoid cells (Soulsby, 1972), and eosinophils (Litt, 1964; Hirsch, 1965) has been demonstrated, using different parasite-host systems, at the intestinal level. Furthermore, it was proved by Wehr and Shalkop (1963) in studies done in the biologic system Ascaridia columbae and pigeons, and by Bindseil (1969, 1970a, 1970b) working with the system Ascaris suum and mice (orally and intravenously infected),

how important the liver is as an immunologic barrier, preventing further migration from invasive larvae, trapping them, and even destroying them. Under those evidences, it can be inferred that both, the intestine and the liver, ought to act as organs of defense against A. columbae migrating larvae in orally infected birds.

In the main experiment of this research (Experiment No. 2), infective L2 A. columbae were spared of confronting the harmful immune reactions at the pigeons intestine and liver, because they were intravenously injected. Infective A. columbae larvae began their life cycle in the host's lungs. How did pigeons face and answer such unheralded infection? How did the route of infection influence the establishment of a patent infection? Were the host's mechanisms of defense able to overcome the infection? These are queries analyzed and discussed later.

Histopathologic studies outlined the progressive cellular reactions and tissue changes at the pulmonary level, trying to halt the infective A. columbae larvae. It should be mentioned that eosinophils, and lymphocytes were the cells generally involved in the defense process. Normal or destroyed larvae were not observed until 24 days post-infection when at the same a few giant cells of foreign body type were seen. Larvae were apparently passed through the lung tissues and air passages, arriving through the trachea to the upper digestive tract. Granuloma and giant cell formation appeared delayed, since those cells were seen 8-10 days post intravenous injection in other pigeons (Roy Melendez, unpublished results). It was reported by Guttman et al. (1971), that the intensity of the humoral immune response of pigeons is of low magnitude, and the

appearance of detectable precipitating and hemagglutinating antibodies is delayed in contrast to the chicken and some mammals.

Larvae collected from pigeon 72 had inflammatory cells adhered to their cuticle, and they were few in number, and almost devoid of movement. These findings could be explained as a secondary activity of inflammatory cells already concentrated in lungs, as a consequence of a heavy fungi infection in that bird. This undiagnosed mycotic infection was also to blame for the apparent significance between larvae collected from lungs of birds 72 (received India ink dose), and 79 (lacking India ink dose). The difference seen in the larval count was attributed to the mycotic infection and not to the single dose of that inert material. The fact that no larvae were collected from the liver suggests, that A. columbae larvae arrived at the small intestine through the trachea. Had larvae migrated from the lungs to the liver through the general circulation, they would have been trapped in the hepatic tissue. This apparently happened in one granuloma seen in liver of pigeon 38; however, another one probably was caused as a reaction against India ink particles. A. columbae larvae established in the intestine, did not migrate toward hepatic tissues as normally occurs in orally infected pigeons.

The administration of parasitic infective stages to a suitable host by different routes of entry, often brings about different degrees of parasitism, variable tissue reactions, and even changes in the pathogenicity of the parasite. Incidentally, attention should be called to the term "suitable host", that is to say, a host able to provide the parasite with adequate food, a comfortable environment, and taking care,

totally or partly, of the parasite's relationships with the external environment. For instance, prior to these experiments, tentative trials were done attempting to infect per os a pair of mice with a high dose of Ascaridia columbae infective eggs (3,600 to 4,000). Mice were not infected (they were not suitable hosts for this avian parasite), and intact embryonated eggs of A. columbae were collected from mouse feces 20 to 30 hours post infection. This can show that hatching was not triggered in the mouse intestine which does not appear to be a suitable environment for embryonated eggs of this avian parasite.

For centuries pigeons have been naturally or experimentally infected with embryonated eggs of A. columbae by the oral route. The administration of artificially hatched L2 A. columbae intravenously is positively an advantage for the nematode, whose larvae are able to survive in extra-intestinal tissues of pigeons (liver, lungs, bloodstream) (Wehr and Hwang 1958, 1959), and because, they did not stimulate nor confront the immune responses of the host at the intestinal or hepatic tissues. Therefore, immature stages of A. columbae were able to arrive in a probably non-sensitized small intestine, either as L2 or L3, to molt to the next stages in the lumen, to increase in body length especially as L4 (Dobson, 1972), and to reach unexpected or untold body length when adults (Graph 1). It was well stated by Soulsby (1961): "In an immune animal infective larvae are able to invade the animal tissues, to persist there for a short while, and even to grow, but they are prevented from reaching the size at which they would normally undergo the molting process. The greater the degree of immunity, the more marked is the effect

on the degree of growth." Dobson (1972) also stated: "A major effect of helminth immunity is retardation of growth and development."

A close examination of graph 1 reveals the following: adult Ascaridia columbae recovered from intravenously infected birds had body length, not only exceeding the body length of adult worms collected for orally infected pigeons, but also well over the measurements considered as "normal" for A. columbae. With these statistically significant results ($P \leq .0001$) it can be suggested that the administration of A. columbae intravenously, coupled with the lack of migration of A. columbae larvae through the gut and liver of the host, brought about A. columbae infected pigeons whose immune system was not able to control the growth of L4 or adult worms at the intestinal level. It seems that a local immune response was not evoked by the functional antigens of infective L2 A. columbae. The theory of the crowding phenomenon (the lower the number of infective helminths, the longer their body would be and vice versa) was thought as another possible reason for explaining the length of A. columbae retrieved from intravenously infected pigeons. However, it was discarded under the presence of the following evidences: (a) Orally infected pigeons (No. 404 and 432) had slight infections, nevertheless worms measured were smaller than those collected from intravenously infected pigeons, but longer than those obtained from orally infected ones with a rather high burden of nematodes (Table 3). (b) Worms collected from pigeon 66 were longer than those retrieved from pigeon 405 (Graph 1). The populations of A. columbae observed in pigeons 66 and 405 were 68 and 355 respectively (Table 1). Evidences pointed out in (a) and (b) can be explained by the crowding phenomenon

theory; however, the unmatched length of adult A. columbae collected from intravenously infected pigeons (Graph 1), should have a reason beyond the crowding phenomenon. It is, therefore, suggested that the immune response of parenterally infected pigeons was not able to cope with the developing nematodes at the intestinal level, which could explain the unmatched length of adult male and female A. columbae.

Studies carried out by Bindseil (1970c) on the biologic system Ascaris suum in mice, assessed the effects of different stimuli of mice upon infection with A. suum. One of his conclusions was that the stimulation of the reticuloendothelial system (RES) by injections of an inert material (indigo carmin) did not influence the mean larval count collected from liver, compared to that of the controls. Pigeons 72, 38, and 37, which received single intravenous injections of India ink 8 days post infection, did not show differences in the total larval counts collected from lungs or intestine. These results duplicated Bindseil's conclusions.

The experiment dealing with evaluation of female A. columbae oviposition in different media, was planned in order to find out if that biologic activity could be modified by homosexual and/or heterosexual maintenance solutions of the same parasite, containing secretory and excretory products (SEP). After Greet (1964) reported that sexual attraction in the nematode Paragrolaimus rigidus was induced by the presence of attractants in the SEP, several papers have been published dealing with the sexual or chemical attraction in adult worms. Roche (1966) studied the phenomenon in the system Ancylostoma caninum of dogs.

Gimenez and Roche (1972) published their results on sexual attraction between male and female Nippostrongyhs brasiliensis in rats. Anya (1976) studied the same phenomenon in Aspiculuris tetraptera, and recently Roberts and Thorson (1977) using adult N. brasiliensis in vivo and in vitro, described the sexual attraction related to the host immunity.

Other aspects on the biology of those attractants, nowadays called pheromones, of animal nematodes have not been studied. To hypothesize that male pheromones could modify the rate of oviposition of female nematodes of the same species does not seem logical since it appears to be against the principle of species survival, nevertheless, it is well known that in natural and experimental infections of other nematodes, males are sooner expelled than females, and in the case of Trichinella infection, males die shortly after copulation (Soulsby, 1968). This male behavior appears to indicate that after females are fecundated, males are not longer needed or for some reasons they are biologically undesired.

Egg counts in saline solution, saline plus antibiotics, and heterosexual or homosexual solutions, seem to yield numerical differences (Graph 2), i.e. 56,100 A. columbae eggs, counted in saline solution during the first 48 hrs., against 23,500 in the male solution after the same time. The fact that only 20 observations were recorded, and that the experiment was not duplicated, caused the lack of a wider statistical analysis. The test used, two way analysis of variance answered that no significant results were detected due to the different treatments used in the test.

The recent discovery made by Roberts and Thorson (1977) that each sex emitts a pheromone attractive to the other, and their statement that males did not release an attractant to other males, leads the author to think that pheromones present in the SEP, could also be responsible for the numerical differences observed in this experiment. Finally, attention should be called to the reality that nematodes kept in saline or other media are dying organisms; therefore, in vitro results may have little relationship to what happens in vivo.

CONCLUSIONS

1. Parasitic infections at the intestinal level were brought about when infective L2 Ascaridia columbae (Gmelin 1790) Travassos 1913, were intravenously injected into susceptible pigeons (Columba livia domestica).
2. Second and/or third-stage A. columbae apparently performed a tracheal migration, and through the upper digestive tract they managed to arrive to the small intestine.
3. Male and female adult A. columbae collected from the alimentary canal of intravenously infected pigeons, had a body length significantly longer ($P \leq .0001$) than those recovered from the intestine of orally infected birds with the same duration of infection.
4. The fact that intravenously administered larvae did not pass through the gut wall and liver of infected pigeons, suggests that migrating larvae arrived to a small intestine devoid of local immune response against A. columbae growing larvae. Nematodes were not controlled by the immune mechanisms of the host, which may explain their unmatched

and unexpected body length.

5. A. columbae-caused granuloma were observed and histophatologically described for the first time in lungs of intravenously infected pigeons.
6. The biologic system of A. columbae and pigeons is honored as a potential model for immunologic studies on migrating ascarids, which are generally carried out on the systems Ascaris suum-mice, A. suum-rabbits, and A. suum-guinea pigs.
7. The levels of oviposition by female nematodes were numerically different when those worms were kept in natural, homosexual, and heterosexual saline media. The largest differences were observed between oviposition rate in the male solution compared to the others. Unfortunately, statistical analysis yielded no significant results.

ABSTRACT

Fully embryonated eggs of Ascaridia columbae (Gmelin 1790) Travassos 1913, kept in suspension in sterile saline solution, were artificially hatched, and free infective larvae were intravenously inoculated in twelve 14-18 week old pigeons (Columba livia domestica). The route of migration and development of A. columbae were followed after infected birds were periodically sacrificed. Thoracic and abdominal organs were analyzed in order to look for inmature or adult stages of the nematode, using the Baermann technique, artificial digestion, and/or histopathologic method.

A. columbae larvae apparently performed a tracheal migration, arriving to the small intestine and establishing a patent infection. Adult worms

collected from the small intestine of intravenously infected birds, showed an unmatched body length, and nematodes were significantly ($P \leq .0001$) longer than adult worms recovered from orally infected birds with the same duration of infection. The absence of migration of those nematodes through the host's gut wall and liver may explain the unexpected body length measured in those worms, since they were not attacked by the cellular and/or humoral immune mechanisms of the host at those organs during their migration.

Experiments with female A. columbae in different media, were oriented to find out if oviposition could be modified by the secretory and excretory products (SEP), probably pheromones, present in homosexual and heterosexual maintenance solutions obtained from the same nematode. Results were numerical different specially between oviposition in "male solution" and saline solution; however, significant differences were not obtained by the statistical methods applied to the data.

ACKNOWLEDGEMENTS

A research is hardly carried out only by one person, behind scene many persons have directly or indirectly cooperated to let me accomplish a thesis like this. I wish I could say thanks to all the anonymous collaborators, I can not, however, in my heart there will always be a "thanks so much" for your generous help. My special gratitude is due to Dr. W. D. Lindquist, my advisor and friend, who guided me to shed this shy beam of light toward the dark and endless tunnel of Science, and to The Universidad Centroccidental, Barquisimeto, Venezuela, and to the

Fondo Nacional de Investigaciones Agropecuarias (FONAIAP), Caracas, Venezuela, for the scholarship and additional funds I was granted with, during the one and a half years I spent obtaining my Master's degree. To Dr. J. West, Diagnostic Lab. Veterinary College, K.S.U. for guiding me to understand the histopathologic lesions found in this research, to Dr. W. Bailie, Microbiology, Dept of Infectious Diseases, K.S.U. for giving me access to his facilities and equipment in order to perform the hatching technique; to Dr. S. M. Gaafar, Purdue University, Lafayette, Indiana, for providing me with a full explanation of in vitro hatching procedures. To Dr. Kemp, Department of Statistics, K.S.U., for the statistic analysis of my data, to Dr. P. B. Khoury, School of Veterinary Medicine, University of Pennsylvania, for his references on hatching techniques, and last but not the least, to my wife Celeste for her help and company during the development of this research.

REFERENCES

1. Ackert, J. E. 1917. A means of transmitting the fowl nematode, Heterakis papillosa Bloch. Science, m.s., 46: 395.

2. _____ 1931. The morphology and life history of the fowl nematode Ascaridia lineata (Schneider). Parasitology 23: 360-379.

3. _____, Cooper, R. M., and Dewhirst, L. W. 1947. Viability of Ascaridia eggs under varying conditions of age and administration. Transactions of the American Microscopical Society. 66: 383-389.

4. _____, Graham, G. L., Nolf, L. D., and Porter, D. A. 1931. Quantitative studies on the administration of variable numbers of nematode eggs (Ascaridia lineata) to chickens. Transactions of the American Microscopical Society. 50: 206-214.

5. Anya, A. O. 1976. Studies on the reproductive physiology of nematodes = the phenomenon of sexual attraction and the origin of the attractants in Aspiculuris tetraptera. International Journal for Parasitology. 6: 173-177.

6. Arean, V. M., and Crandall, C. A. 1962. The effect of immunization on the fate of injected second stage Ascaris lumbricoides larvae in the rabbit. American Journal of Tropical Medicine and Hygiend. 11: 369-379.

*7. Bedel, V.D.C. 1902. Heterakis maculosa dans le foie du pigeon. Bulletin Society of Central Med. Vet. 56: 147-150.

8. Bindseil, E. 1969. Immunity to Ascaris suum. 2. Investigations of the fate of larvae in immune and nonimmune mice. Acta path. microbiol. scand. 77: 223-234.

9. _____ 1970a. Immunity to Ascaris suum. 3. The importance of the gut for immunity in mice. Acta path. microbiol. scand. Section B. 78: 183-190.

10. _____ 1970b. Differences in the migration of Ascaris suum larvae in conventional and in SPF-mice. Acta path. microbiol. scand. Section B. 78: 112-114.

11. _____ 1970c. Immunity to Ascaris suum. 4. The effects of different stimulations upon challenge with Ascaris suum in mice. Acta path. microbiol. scand. Section B. 78: 191-195.

12. Copeman, D. B., and Gaafar, S. M. 1972. Sequential development of hepatic lesions of ascaridosis in colostrum deprived pigs. Australian Veterinary Journal. 48: 263-268.

13. Cram, E. B. 1927. Bird parasites of the nematode suborders Strongylata, Ascaridata, and Spirurata. U.S. National Museum Bulletin. 140, 465 p.

14. Crandall, C. A., Echeverria, R., and Arean, V. M. 1963. Location of antibody binding sites in the larvae of Ascaris lumbricoides var. suum by means of fluorescent techniques. Experimental Parasitology. 14: 296-303.

15. Crompton, D.W.T. and Nehsseim, M. C. 1976. Host-parasite relationship in the alimentary tract of domestic birds. Advances in Parasitology. 14: 96-194.

16. Dobson, C. 1972. Immune response to gastrointestinal helminths. In "Immunity to animal parasites" (E.J.L. Soulsby, ed.), p.p. 191-222. Academic Press, New York and London.

*17. Dujardin, F. 1845. Historie naturelle des helminths ou vers intestinaux. Paris.

18. Fairbairn, D. 1961. The in vitro hatching of Ascaris lumbricoides eggs. Canadian Journal of Zoology. 39: 153-162.

19. Frick, L. P., and Ackert, J. E. 1948. Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. Journal of Parasitology. 34: 192-206.

20. Froyd, G., and Round, M. C. 1959. Infection of cattle with Cysticercus bovis by the injection of onchospheres. Nature (London) 184: 1510.

21. Gemmel, M. A. 1969. Immunological responses of the mammalian host against tapeworm infections. X. Immunization of sheep against Taenia hydatigena and T. ovis with chemically or physically treated embryos. Experimental Parasitology. 26: 58-66.

22. Gimenez, A., and Roche, M. 1972. Influence of male and female Nippostrongylus brasiliensis on each other's distribution in the intestine of rats. Parasitology 64: 305-310.

23. Greet, D. N. 1964. Observation on sexual attraction and copulation in the nematode Panagrolaimus rigidus (Schneider). Nature (London) 204: 96-97.

24. Guttman, R. H., Tebo, T., Edwards, J., Barboriak, J., and Fink, J. N. 1971. The immune response of the pigeon (Columba livia). The Journal of Immunology. 106: 392-396.

25. Hansen, M. F., Oonyawongse, H., and Ackert, J. E. 1953. Rate of development, viability, vigour and virulence of Ascaridia galli ova cultured respectively in air and in water. Amer. Midl. Nat., 49: 743-750.

26. Hansen, M. F., Terhaar, C. J., and Turner, D. S. 1956. Importance of the egg shell of Ascaridia galli to the infectivity of its larva. Journal of Parsitology. 42: 122-125.

27. Herd, R. P., and McNaught, D. J. 1975. Arrested development and the histotropic phase of Ascaridia galli in the chicken. International Journal for Parasitology. 5: 401-406.

28. Hirsch, J. G. 1965. In "The inflammatory process." (B. W. Zweifach L. Grant, and R. T. McCluskey, eds.). p. 266. Academic Press, New York and London.

29. Horton-Smith, C., and Long, P. L. 1956. The anthelmintic effect of three piperazine derivatives on Ascaridia galli (Schrank, 1788). Poultry Science. 35: 606-614.

30. _____, Long, P. L., and Lee, D. L. (1968). Observations on the tissue and post tissue larval development of Ascaridia dissimilis, Vigueras 1931 and a comparison with that of Ascaridia galli in turkeys. Parasitology. 58: 709-714.

31. Hwang, J. C., and Wehr, E. E. 1958. Observations on early development of Ascaridia columbae in the pigeon. Journal of Parasitology. 44 (Suppl.): 26.

32. Ikeme, M. M. 1970. Retarded metamorphosis in larvae of Ascaridia galli following repeated challenge of poultry with infective eggs. Veterinary Record. 87: 725-726.

33. Irwin-Smith, V. A. 1920. Nematode parasites of the domestic pigeon (Columba livia domestica) in Australia. Proceedings Linnean Society of New South Wales. 45: 552-563.

34. Jaskoski, B. J., and Colucci, A. V. 1964. In vitro hatching of Ascaris suum eggs. Transactions of the American Microscopical Society.

35. Johansson, S.G.O., Mellbin, T., and Vahlquist, Bo. 1968. Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND). The Lancet. 1: 1118-1121.

36. Johnson, J. R., Hansen, M. F., and Nassar, R. F. 1974. Ascaridia galli: effects of immunosuppressive drugs or bursectomy in chickens. Experimental Parasitology. 35: 337-349.

37. Levine, H.S., and Silverman, P. H. 1969. Cultivation of Ascaris suum larvae in supplemented and unsupplemented chemically defined media. The Journal of Parasitology. 55: 17-21.

38. Lickley, L., and Monkhouse, F. C. 1962. Development of lipemia in rats following intravenous injection of India ink. Proceedings of the Society of Experimental Biology and Medicine. 103: 777-780.

39. Lindquist, W. D. 1963. Early infections of Ascaridia columbae and Capillaria obsignata in squabs. The Journal of Parasitology. 49: 208.

40. Litt, M. 1964. Eosinophils and antigen-antibody reactions. In: "Annales of the New York Academy of Science" 116: 964-985.

41. Mabon, J. L., and Reid, W. M. 1973. Tissue stages in the development of Ascaridia numidae from the guinea fowl (Numidia meleagridis). The Journal of Parasitology. 59: 1012-1015.

42. Michel, J. F. 1974. Arrested development of nematodes and some related phenomena. Advances in Parasitology, Vol. 12: 279-366. Academic Press, New York and London.

43. Moran, J. F., and Mizelle, J. D. 1956. Notes on the habitat and tissue-phase of Ascaridia galli (Schrank, 1788) The Journal of Parasitology. 42 (Supplement), 18.

44. Mozgovoi, A. A. 1953. Ascaridata of animals and man and the diseases caused by them. Part 1. In: "Essentials of Nematodology," (Skrjabin, K. I., ed.). Vol. II. p.p. 40-241.

45. Ogilvie, B. M., and Jones, V. E. 1971. Parasitological Review. Nippo strongylus brasiliensis: a review of immunity and the host/parasite relationship in the rat. Experimental Parasitology. 29: 138-177.

46. Roberts, T. M., and Thorson, R. 1977. Chemical attraction between adults of Nippostrongylus brasiliensis: description of the phenomenon and effects of host immunity. The Journal of Parasitology. 63: 357-363.

47. Roche, M. 1966. Influence of male and female Ancylostoma caninum on each other's distribution in the intestine of the dog. Experimental Parasitology. 19: 327-331.

48. Roneus, O. (1966). Studies on the aetiology and pathogensis of white spots in the liver of pigs. Acta Veterinaria Scandinavica. 7: suppl. 16, 1-112.

49. Salvidio, E., Crosby, W. H. 1960. Thrombocytopenia after intravenous injection of India ink. Journal of Laboratory and Clinical Medicine. 56: 711-716.

50. Sharma, N. N., and Reid, M. W. 1962. Successful infection of chickens after parenteral inoculation of oocysts of Eimeria sp. The Journal of Parasitology. 48 (Suppl.): 33.

51. Soulsby, E. J. L. 1961. Some aspects of the mechanism of immunity to helminths. Journal of the American Medical Association. 138: 355-362.

52. Soulsby, E. J. L. 1968. Helminths, arthropods and protozoa of domestic animals (Mönning). 6th edition. Baltimore, The Williams and Wilkins Company. pp. 314-317.

53. _____ 1972. Cell-mediated immunity responses in parasitic infections. In: "Immunity to animal parasites," (E. J. L. Soulsby, ed.), pp. 57-95. Academic Press, New York.

54. Sprent, J. F. A. 1959. In: "The evolution of living organisms" (G. S. Leeper, ed.), pp. 149-165. Melbourne University Press, Melbourne, Australia.

55. Tugwell, R. L., and Ackert, J. E. 1952. On the tissue phase of the life cycle of the fowl nematode Ascaridia galli (Schrank). The Journal of Parasitology. 38: 277-288.

56. Urquhart, G. M. 1965. Parenteral production of Cysticercosis. The Journal of Parasitology. 51: 544.

57. Ward, K. A., and Fairbairn, D. 1972. Chitinase in developing eggs of Ascaris suum (Nematoda). The Journal of Parasitology. 58: 546-549.

58. Wehr, E. E. 1971. Parasitic infections. In: "Infectious and parasitic diseases of wild birds," (Davis, J. W., ed.) The Iowa State University Press. Ames, Iowa, pp. 198-200.

59. _____, and Colglazier, M. L. 1968. Thiabendazole as an anthelmintic against Ascaridia columbae in pigeons. Proceedings of the Helminthological Society of Washington. 35: 117-118.

60. _____, and Hwang, J. C. 1959. Further observations on the life history and development of Ascaridia columbae (Gmelin, 1790) Travassos, 1913, in the pigeon. The Journal of Parasitology. 45(Suppl.): 43.

61. _____ 1964. The life cycle and morphology of Ascaridia columbae (Gmelin, 1790) Travassos, 1913 (Namatoda: Ascarididae) in the domestic pigeon (Columba livia domestica). The Journal of Parasitology. 50: 131-137.

62. _____ and Shalkop, W. T. 1963. Ascaridia columbae infection in pigeons: a histopathologic study of liver lesions. Avian Diseases. 7: 206-211.

---

*Original publication not seen.

STUDIES ON *ASCARIDIA COLUMBAE*: EXPERIMENTAL LIFE CYCLE IN PARENTERALLY INFECTED PIGEONS, AND FACTORS AFFECTING THE OVIPOSITION *IN VITRO*

by

ROY DANIEL MELENDEZ,
DVM., Universidad Centro Occidental, 1970
Barquisimeto, Venezuela

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCES

Department of Infectious Diseases

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1977

Fully embryonated eggs of Ascaridia columbae (Gmelin 1790) Travassos 1913, kept in suspension in sterile saline solution, were artificially hatched, and free infective larvae were intravenously inoculated in twelve 14-18 week old pigeons (Columba livia domestica). The route of migration and development of A. columbae were followed after infected birds were periodically sacrificed. Thoracic and abdominal organs were analyzed in order to look for inmature or adult stages of the nematode, using the Baermann technique, artificial digestion, and/or histopathologic methods.

A. columbae larvae apparently performed a tracheal migration, arriving to the small intestine and establishing a patent infection. Adult worms collected from the small intestine of intravenously infected birds, showed an unmatched body length, and nematodes were significantly ($P \leq .0001$) longer than adult worms recovered from orally infected birds with the same duration of infection. The absence of migration of those nematodes through the host's gut wall and liver may explain the unexpected body length measured in those worms, since they were not attacked by the cellular and/or humoral immune mechanisms of the host at those organs during their migration.

Experiments with female A. columbae in different media, were oriented to find out if oviposition could be modified by the secretory and excretory products (SEP), probably pheromones, present in homosexual and heterosexual maintenance solutions obtained from the same nematode. Results were numerical different specially between oviposition in "male solution" and saline solution; however, significant differences were not obtained by the statistical methods applied to the data.