11-1982

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A Thesis
Submitted To The Graduate Faculty Of
The University of Richmond

In Candidacy
For The Degree Of
Master of Science
In Biology

November, 1982

Reproductive Biology of the Rat Lungworm

Angiostrongylus cantonensis

(Metastrongyloidea: Angiostrongylidae)

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Acknowledgments

I wish to thank my major advisor, Dr. Thomas R. Platt, for stimulating my initial interest in parasitology, and for his guidance in the preparation and writing of this thesis. I also wish to thank Dr. J. Van Bowen and Chris Gennings for their help in the preparation of statistics. Thanks are extended to Prudence Huddleston for her artistic assistance with graphs.

In addition, thanks are expressed to Dr. Kathryn J. Schneider and to Dr. William S. Woolcott for their critical reading of the manuscript.

Abstract

Twenty rats (Rattus norvegicus) received infective inocula of 5, 10, 20, and 40 third-stage larvae of Angiostrongylus cantonensis. The effects of varying doses of larvae on prepatent period, larval production, worm establishment, worm size, and fertility were studied. The aquatic gastropod Biomphalaria glabrata was the experimental intermediate host.

The duration of the prepatent period varied from 37 to 45 days. The shortest prepatent period was observed in animals that received the highest infective inoculum. The prepatent period showed a significant inverse relationship with increasing inoculum size. Larval production increased logarithmically, followed by a plateau phase. The number of larvae per gram of feces (52,000 and 40,000) was highest in animals receiving infections of 20 or 40 larvae, respectively. The percentage establishment of worms in rats increased as infection levels increased. Effects of crowding on A. cantonensis were not observed in females; however, a significant positive relationship in body volume was observed for males. There was no relationship between fertility (number of larvae per gram of feces per female) and population density.

The Metastrongyloidea are bursate nematodes and extraintestinal parasites of mammals. The majority of these organisms parasitize the lungs as adults; however, some species inhabit non-pulmonary sites, and a few are neurotropic. The metastrongylids require an intermediate host, generally an annelid or terrestrial gastropod (Anderson, 1968). Some species may employ a vertebrate or invertebrate transport host.

Species of the Angiostrongylidae are a common component of the helminth fauna of marsupials, insectivores, carnivores, and rodents (Anderson, 1968). They typically utilize gastropods as intermediate hosts. The family consists of 16 genera, only one of which, Angiostrongylus Kamensky, 1905, has been demonstrated to be of any medical importance.

Angiostrongylus cantonensis (Chen, 1935) was first observed in the lungs of an undescribed species of Rattus in Canton, China by Chen (1933). Chen (1935) later described this organism as Pulmonema cantonensis. Dougherty (1946) synonymized Pulmonema with Angiostrongylus, and hence the valid name of this species is Angiostrongylus cantonensis (Chen, 1935).

Angiostrongylus cantonensis is typically a parasite of rats. Females deposit eggs in the pulmonary arteries. Eggs become trapped in the capillaries of the lungs where development and hatching occur. First stage larvae (L1) migrate up the trachea, are swallowed and expelled in the feces (MacKerras and Sandars, 1955).

A large number of aquatic and terrestrial gastropods serve as suitable intermediate hosts of this nematode (for a review see Anderson, 1968). The L1 mature to third-stage larvae (L3) within the tissues of the mollusc. Rats become infected by ingesting infected snails.

Upon ingestion, most L3 migrate to the liver of the rat via the portal vein, and subsequently travel to the posterior vena cava (MacKerras and Sandars, 1955). The larvae enter the right atrium of the heart, then they migrate to the lungs by way of the pulmonary artery and ultimately reach the main circulation. Some L3 gain entrance to the central nervous system, complete two molts in the brain, becoming pre-adults. Approximately 27 days post-infection, young adults leave the brain and migrate to the heart and lungs (MacKerras and Sandars, 1955; Alicata, 1965). They reach their final destination, the pulmonary arteries, where sexual maturity is attained in approximately seven days, followed by fertilization. Eggs are then deposited in the lung parenchyma. The prepatent period, the time between the administration of the infective inoculum to the definitive host and the initial discovery of L1 in

the feces, has been established at 42 to 45 days (MacKerras and Sandars, 1955; Alicata, 1965).

Organisms belonging to the Angiostrongylidae received little attention until A. cantonensis was demonstrated to be the causative agent of eosinophilic meningioencephalitis in man (Alicata, 1962). Once the relationship between A. cantonensis and this zoonotic disease was determined, angiostrongylid research increased dramatically (Rosen et al., 1961: Vaillant et al., 1961: Wallace and Rosen, 1969).

Angiostrongylus cantonensis was not reported in man until 1945 (Nomura and Lin, 1945) when young adult lungworms were found in the cerebrospinal fluid of a man suffering from meningitis in Taiwan. Since this finding, A. cantonensis larvae have been studied closely, and the roles of paratenic and intermediate hosts considered (Alicata, 1962; Rosen et al., 1961 and Vaillant et al., 1961). Acquisition of infection by man may follow the ingestion of insufficiently cooked snails, freshwater prawns, or land crabs. Contaminated drinking water and fresh vegetables also may be a source of human infection (John and Martinez, 1975). Following ingestion, L3 migrate from the gastrointestinal tract to the central nervous system. Ten days post-infection larvae are present in the brain. The larvae provoke a severe acute inflammatory reaction consisting primarily of eosinophilic polymorphonuclear leukocytes. The resulting disease in man, eosinophilic meningioencephalitis, has been reported in parts of Southeast Asia and in the Pacific

Islands (Alicata, 1965).

Larval stages of A. cantonensis have been found in natural infections in a variety of species of the primarily terrestrial pulmonate order Stylommatophora (Anderson, 1968). Some aquatic molluscs (Order: Bassomatophora) have also been successfully infected experimentally (e.g., Richards and Merritt, 1967).

Research on A. cantonensis has concentrated principally on the life history (MacKerras and Sandars, 1955; Bhaibulaya, 1975). Many aspects of the life cycle, however, are still not fully understood. The relationship between the length of the prepatent period and the size of the infective inoculum has not been established. Other aspects of the life cycle of A. cantonensis such as the rate of establishment of infective larvae in the definitive host, and the reproductive capacity of the adult females have not been assessed. This information could contribute substantially to our understanding of the transmission and epizootiology of this parasite.

The following aspects of the life cycle were examined.

1. Confirm the length of the prepatent period for A. cantonensis in rats.

2. Determine if a relationship exists between the size of the infective inoculum (L3) and the length of the prepatent period.

3. Examine the establishment of third-stage larvae and the sex ratio of the adults in populations of A. cantonensis.

4. Determine if a relationship exists between the size of the infective inoculum (L3) and the number of first-stage larvae in the feces.

5. Determine the effects of population density on worm size and fertility.

Materials and Methods

Twenty male, laboratory rats (Rattus norvegicus) were obtained as juveniles (100 to 150 grams) from Dominion Laboratories Incorporated, Dublin, Virginia. Animals were maintained in individual wire mesh cages and fed Purina Rat Pellets and water ad libitum. All animals were maintained under conditions that would prevent contact with the infective stages of Angiostrongylus cantonensis.

Infective larvae (L3) of A. cantonensis were obtained from experimentally-infected Biomphalaria glabrata (Gastropoda: Bassomatophora). Third-stage larvae were removed from snails by artificial digestion. Snails were removed from the shell, minced with fine scissors, and placed in a test tube containing approximately ten ml of digestion fluid (0.6g pepsin and 0.7ml HCl/100ml distilled water). The test tubes were incubated in a water bath at 37C ± 1C for two or three hours. Test tubes were subsequently centrifuged at high speed (2000RPM) for four minutes. Two ml of supernatant and the residual plug were examined for

larvae using a dissecting microscope. The larvae (L1) were counted and placed in physiological saline prior to infecting rats.

Four groups of five rats each were given various inocula of L3 (Table I). Rats were lightly anesthetized with ether and infected orally. Saline containing larvae was pipetted into the rear of the mouth, stimulating the gag reflex, forcing the animals to swallow. Additional saline was administered to assure the removal of all larvae from the pipette.

Fecal examinations were initiated approximately 30 days post-exposure (PE) and continued daily for three weeks following the appearance of larvae in the feces. Feces were then examined every third day until the animal was killed.

Feces were examined for first-stage larvae using the Baermann technique. Fresh feces were collected every 24 hours on wire screens and examined the following day. Fecal pellets were weighed to the nearest 0.1g and placed on a wire mesh platform in a 13cm diameter funnel. One-hundred fifty ml of distilled water, sufficient to cover the feces, was then added. The suspension was allowed to stand overnight (12 hrs).

At collection, 15ml of Baermann fluid was removed through a clamped rubber hose at the bottom of each funnel into a centrifuge tube. The tubes were centrifuged at 2000RPM for five minutes. The top 13ml of supernatant were

removed using a vacuum apparatus. The remaining two ml were mixed and poured into a counting dish. Larvae were counted using a Wild dissecting microscope.

At 39 days post-patency rats were killed with a lethal dose of sodium pentabarbital administered intraperitoneally. The lungs and heart were removed from each rat and placed in a petri dish containing saline. The pulmonary arteries were carefully examined for the presence of adult worms.

Worms were killed in glacial acetic acid and stored in glycerine alcohol (95 parts 70% ethanol and 5 parts glycerine). They were subsequently cleared for microscopic examination in lactophenol. Worms were measured with the aid of a drawing tube, measuring wheel, and stage micrometer. The volume of nematodes was determined by measuring the length and width of the worms and calculating the volume of a cylinder ($V = \pi r^2 h$).

Moving averages were calculated for larval output data. Two-way analysis of variance (ANOVA) was used to compare prepatent period and larval production with the size of the infective inoculum. Linear regression was performed to determine the effects of population density on worm size and fertility. Two-way student's t-test was used to analyze differences between infective inoculum and female establishment. Analyses were done using the Statistical Package for the Social Sciences (SPSS) on a VAX 11/750 digital computer. Results were considered significant if $p \leq 0.05$.

Results

The duration of the prepatent period varied from 37 to 45 days (Table I). The shortest prepatent period was observed in animals that received the highest infective inocula. The prepatent period shows a significant negative relationship with increasing inoculum size ($F = 15.44$; $p < 0.01$; Fig. 1).

Moving averages of larval production increased logarithmically from the second to the seventh day of patency, followed by a plateau phase (Fig. 2). Rats infected with five and 10 larvae (Groups 1 and 2) had peak larval outputs of 460 and 2200 larvae per gram (LPG), at 10 and 12 days post-patency (PP), respectively. Peak larval production in rats infected with 20 L3 (Group 3) was 52,000 LPG at seven days PP. Peak larval production in rats that received 40 larvae (Group 4) was 40,000 LPG and occurred 11 days PP.

No significant difference was found in larval production between Groups 1 and 2 or between Groups 3 and 4. Groups 1 and 2, however, did have a significantly lower larval output than Groups 3 and 4 ($F = 11.15$; $p < 0.0001$).

A total of 253 worms was found at necropsy. Two-hundred forty four worms (96%) were found in the pulmonary arteries, and 4% were found in the heart. The percent establishment of adult worms in the pulmonary circulation increased with increasing infective inoculum. The mean

Table I Length of Prepatent Period vs. Inoculum Size for A. cantonensis in the Rat (Rattus norvegicus)

Group	n	No. of larvae in infective inoculum	$\bar{X}$ prepatent period $\pm$ S.D.*
1	5	5	44.7 $\pm$ 0.58 **
2	5	10	43.0 $\pm$ 0.82 ***
3	5	20	38.6 $\pm$ 0.55
4	5	40	37.6 $\pm$ 0.89

* days

** two animals did not become patent

*** one animal did not become patent

Figure 1. Regression analysis of the inoculum size versus the duration of the prepatent period in rats experimentally infected with A. cantonensis.

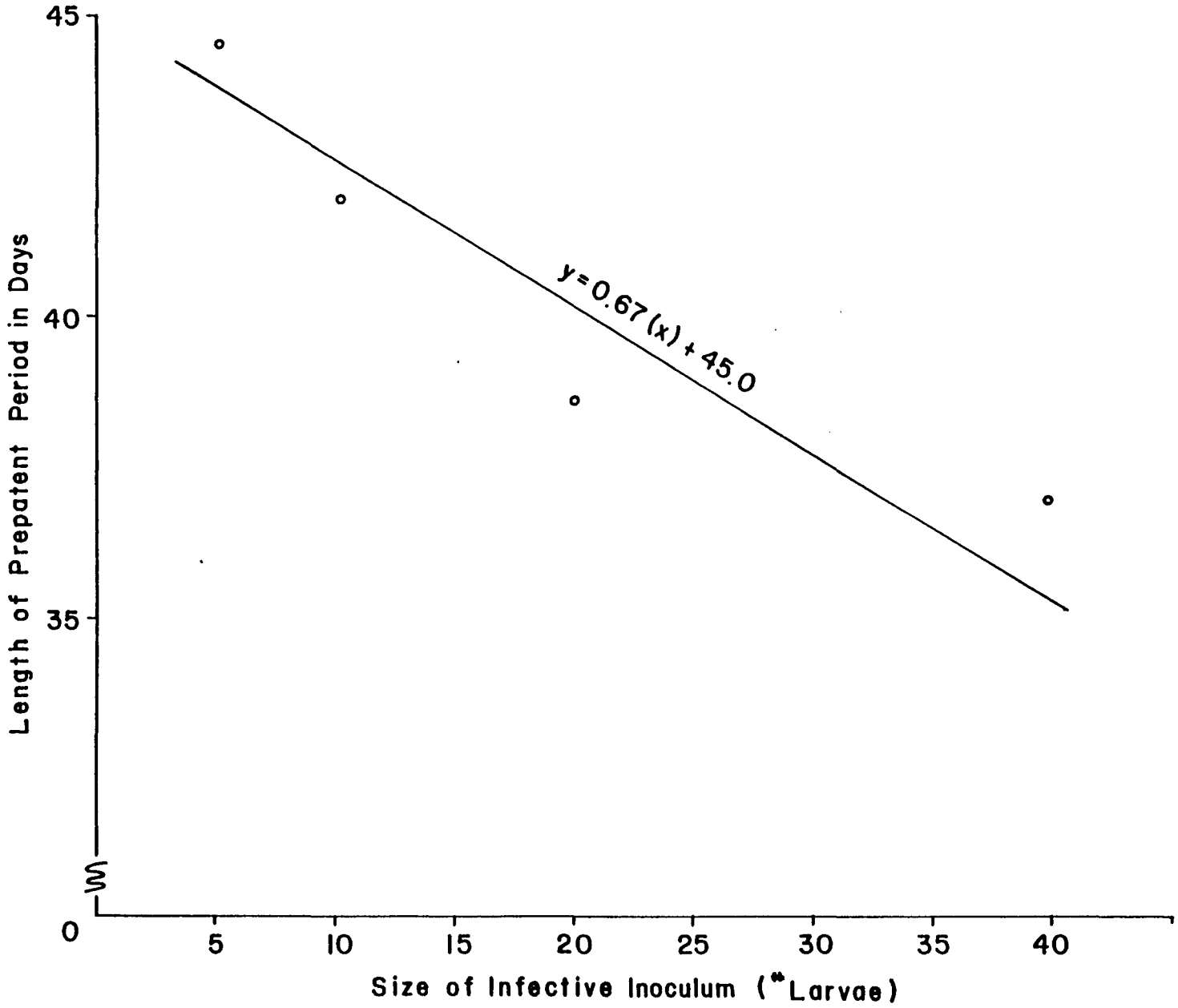
45
40
35
0
Length of Prepatent Period in Days
y=0.67(x)+45.0
5
10
15
20
25
30
35
40
Size of Infective Inoculum (** Larvae)

FIGURE 1

Figure 2. Numbers of first-stage larvae recovered from rats experimentally infected with 5, 10, 20, and 40 infective larvae of A. cantonensis.

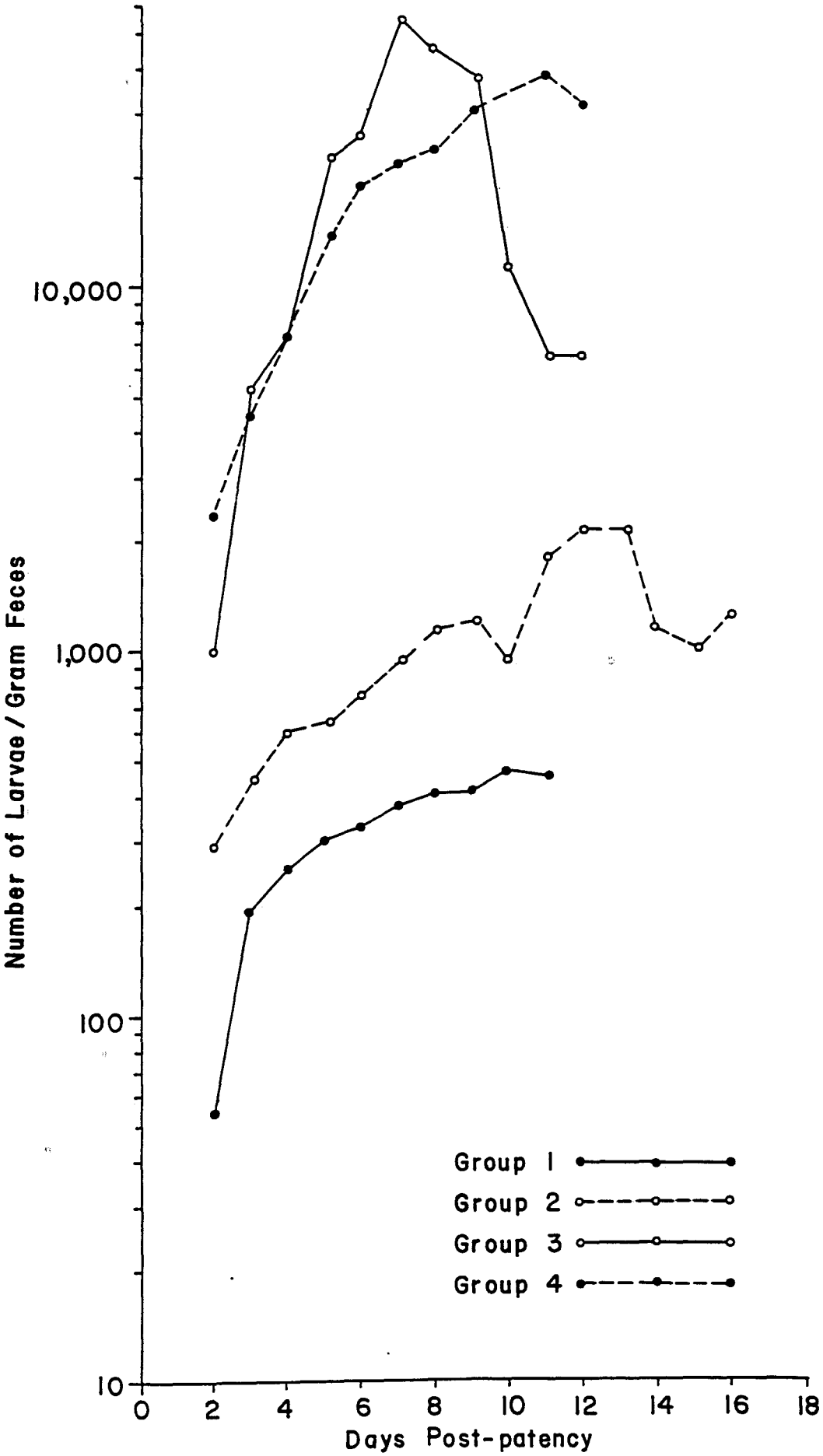
10,000
1,000
100
10
Number of Larvae / Gram Feces
0
2
4
6
8
10
12
14
16
18
Days Post-patency
Group 1
Group 2
Group 3
Group 4

FIGURE 2

number of males and females found in rats was recorded and values are given in Table II. The difference in the number of females in Groups 1 and 2 was not significant. Significant differences were found, however, between the number of females present in Group 3 and Group 4 ($t = 2.41$: $p < 0.05$) and between Groups 1 and 2, and 3 and 4 ($t = 4.01$: $p < 0.001$). The sex ratio (males/females) was 1:1 at all levels of infection. The only major deviation was found in Group 1, which received the lowest infective inoculum (1:3). Adult worms were not found in two rats that were infected with five L3. Two rats infected with 40 L3 yielded 42 worms at necropsy.

Adult females were significantly larger than males, having mean volumes of approximately $5.4mm^3$ and $2.0mm^3$, respectively. Regression analysis (Fig. 3) showed no relationship between mean worm volume and population density for female worms. A significant positive relationship, however, existed for males ($F = 19.95$; $p < 0.001$).

Fertility was calculated as mean number of larvae per gram of feces per female for each rat over all days. The results of a linear regression analysis (Fig. 4) indicated no relationship between fertility and number of worms present.

Table II. The Establishment and Sex Ratio of A. cantonensis in the Pulmonary Circulation of the Rat.

Group	n	# L3/rat ($\bar{X}$)	% Establishment	$\bar{X}$ No. Males ± S.D.	$\bar{X}$ No. Females ± S.D.	sex ratio male/female
1	5	3.3	50	0.6 ± 0.5	2.0 ± 1.6 *	1:3.3
2	5	6.2	60	2.4 ± 1.4	2.8 ± 1.7	1:0.9
3	5	15.0	65	6.0 ± 3.5	7.0 ± 3.9 **	1:1.2
4	5	31.8	75	15.4 ± 7.5	14.8 ± 6.6 &&	1:1.0

n = # rats/group

* Groups 1 and 2 not significantly different

** Group 4 significantly greater than Group 3

&& Groups 3 plus 4 significantly greater than Groups 1 plus 2

Figure 3. Regression analysis of mean worm volume for male and female A. cantonensis versus the total population size.

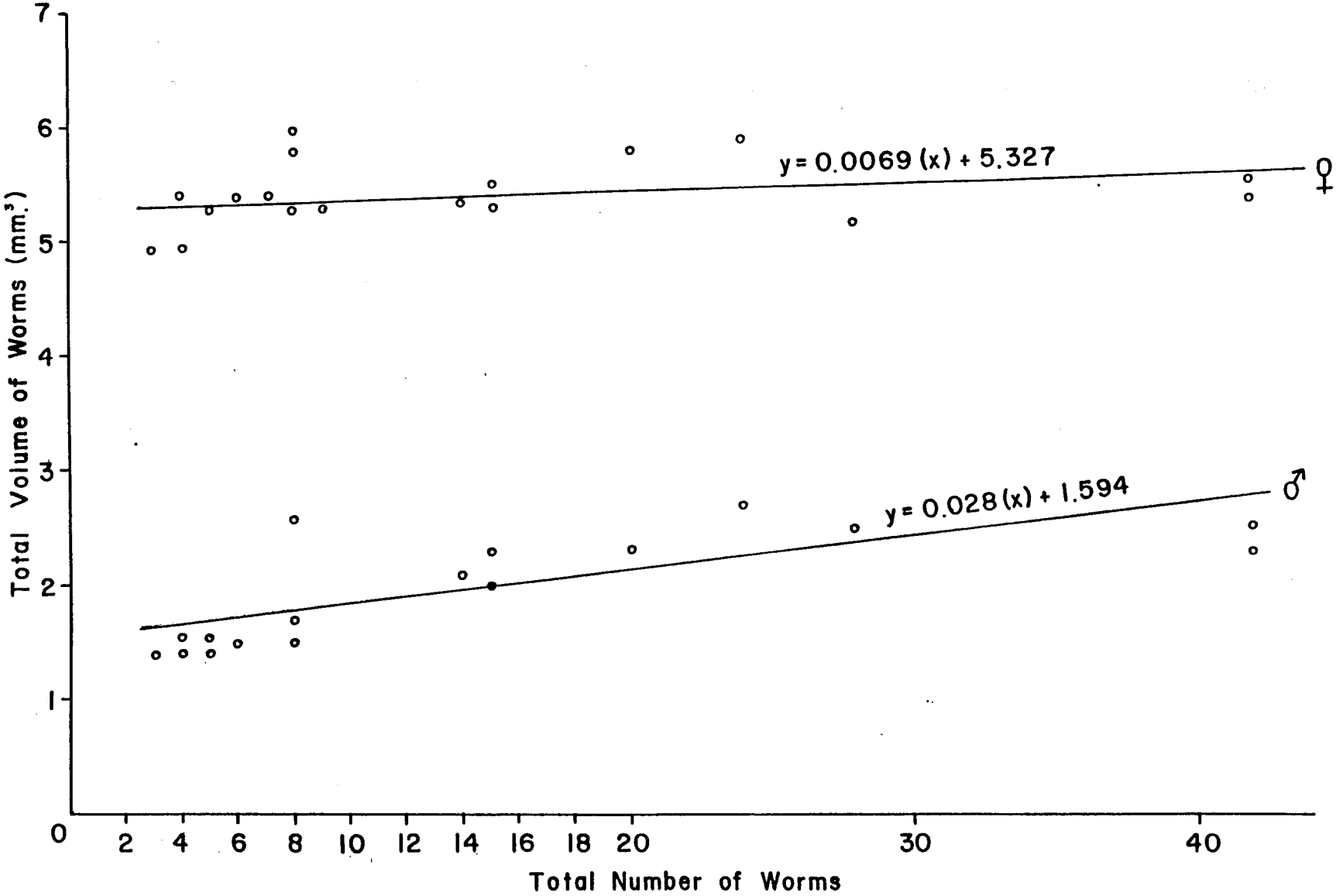
Total Volume of Worms (mm.³)
Total Number of Worms
y = 0.0069 (x) + 5.327
♀
y = 0.028 (x) + 1.594
♂
0
1
2
3
4
5
6
7
2
4
6
8
10
12
14
16
18
20
30
40

FIGURE 3

Figure 4. Regression analysis of worm fertility (number of larvae released per gram of feces per female) versus population size. Arabic numbers represent the number of observations at a single point.

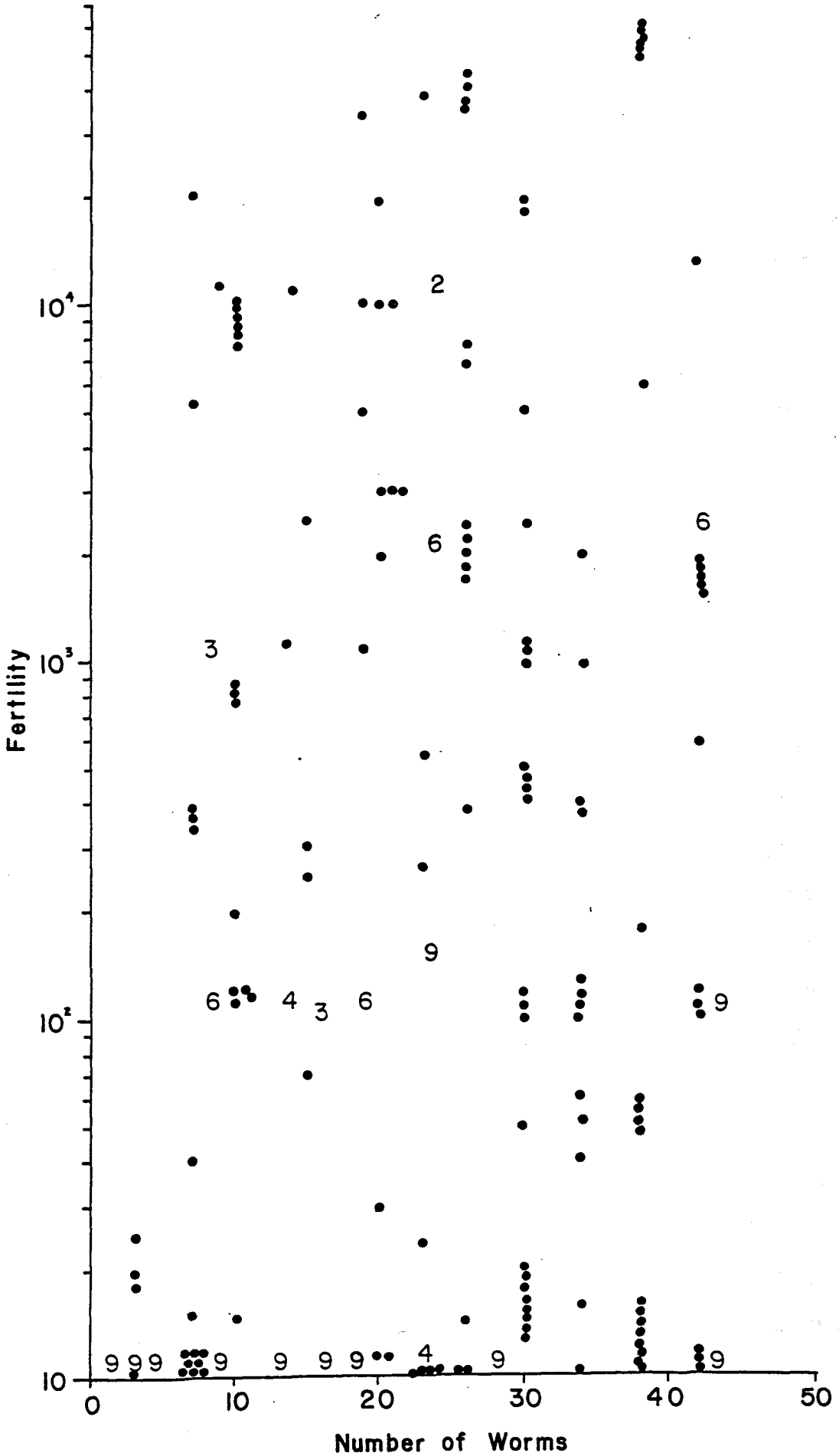

FIGURE 4

Discussion

Reports of the length of the prepatent period for A. cantonensis in the rat have ranged from 40 to 48 days (MacKerras and Sandars, 1955; Alicata and Jindrak, 1970; Bhaibulaya, 1975). The primary focus in these early works was on the life history of the organism and hence, qualitative rather than quantitative observations were stressed. Whole infected slugs were orally administered to rats to enhance tracking of the larval migration (MacKerras and Sandars, 1955; Alicata, 1965; Alicata and Jindrak, 1970). In the present study, inoculum size was controlled to assess its influence on prepatency.

A range in prepatent period from 37 to 45 days post-infection (PI) was observed in the present study. The shortest prepatent period reported in the literature for A. cantonensis in the same host was 40 days (Alicata and Jindrak, 1970). Most investigators report a prepatent period in excess of 40 days; 42-45 days (Bhaibulaya, 1975); 42-48 days (MacKerras and Sandars, 1955; Alicata, 1965). A prepatent period of 37 days is the shortest period of time in which first-stage larvae of A. cantonensis have been observed in the feces of the definitive host. The shortened prepatent period may be the result of a procedure modification used in the present study. The sensitivity of the Baermann technique was enhanced by concentrating the Baermann fluid via centrifugation. Low numbers of larvae

were detected in the concentrate, and a shorter prepatent period resulted. Other investigators (Alicata, 1965; Bhaibulaya, 1975) do not mention concentrating Baermann fluid and consequently the procedure was less sensitive to the detection of low numbers of L1 and hence may have resulted in slightly longer prepatent periods.

There was a significant inverse relationship between the size of the infective inoculum and prepatent period (Fig. 1). Platt and Samuel (1978) observed a similar relationship in the protostrongyloid, Parelaphostrongylus odocoilei (Metastrongyloidea: Protostrongylidae). The prepatent period for P. odocoilei in the mule deer, Odocoileus h. hemionus, ranged from 40 to 62 days with 334 and 14 L3, respectively. A threshold of 100 infective larvae was necessary to achieve a significantly reduced prepatent period (Platt and Samuel, 1978). These authors, however, failed to demonstrate a similar relationship in black-tailed deer (Odocoileus h. columbianus).

Dioecious parasites, when present at low densities, require a prolonged search period between males and females (Kennedy, 1976). This search period may be responsible for the prolonged prepatent period observed for A. cantonensis at low densities. MacDonald (1965) reported that the extent of mating in trematode populations (Schistosoma spp.) depends on the number of worms in any individual host. Assuming the invasion of male and female larvae to be equal a population containing four schistosomes will have a 0.625

probability of pairing, whereas the probability rises to 0.96 in a population of 400 worms. Ulmer (1971); however, reported that light infections with the infective stages of various helminths resulted in patent infections. This might be the result of the release of pheromones (Bone, 1982), or by the chance meeting of preadult or adult worms at the same location (Kennedy, 1976).

The variability in the timing of migration to the site of maturation for low and high density infections may also influence mating probabilities in lungworm populations (Kennedy, 1976). For copulation to occur, males and females must settle in the same part of the lung capillaries at the same time. This becomes less probable in low level infections. A number of worms of opposite sex in the same bronchiole or bronchus is common in high density infections however, and the chances of successful copulation are high provided the worms remain in the lung long enough to attain full sexual maturity (Kennedy, 1976). The expulsion of worms from the lungs by the flow of mucus prior to the attainment of sexual maturity has been reported, however, for Metastrongylus elongatus infections in domestic pigs (MacKenzie, 1959).

Dhar and Sharma (1978) studied the effects of inoculum size and length of prepatent period of Dictyocaulus filaria (Trichostrongyloidea: Dictyocaulidae) in lambs. They found no significant differences in the length of the prepatent period using inocula ranging from 100 to 300 larvae. The

massive number of larvae used by Dhar and Sharma (1978) may account for the absence of a relationship between prepatent period and inoculum size. The large numbers of larvae used in this work may surpass a critical threshold inoculum above which longer prepatent periods are undetectable. Large inocula will obviously reveal only the shortest prepatent periods, and consequently a relationship between infective inoculum and prepatent period would be masked. A wide range of infective inocula must be used to detect these relationships. A critical threshold of 40 larvae was necessary to achieve the shortest prepatent period for A. cantonensis in the present study. The prepatent period may be even shorter if higher infective inocula are used and do not prove lethal to the host.

There are no data in the literature regarding larval production for species of Angiostrongylus. Larval output over time is consistent with that demonstrated for other metastrongyloids, Parelaphostrongylus andersoni by Nettles and Prestwood (1976) and Parelaphostrongylus odocoilei by Platt and Samuel (1978). The curve consists of a logarithmic, plateau, and decline phase (Fig. 2). Immediately following patency, Nettles and Prestwood (1976) reported a rapid increase in larval production for P. andersoni in the white-tailed deer. Following the log phase, larval production declined from 5-7 weeks post-patency (PP). Platt and Samuel (1978) reported a logarithmic increase for P. odocoilei in the black-tailed deer during the first 2-3 weeks of

patency, and then larval production declined sharply 4-7 weeks PP. Panin (1964) described a similar situation for Elaphostrongylus panticola (= E. cervi panticola) in the maral deer (Cervus elaphus maral); however, the initial rise in larval production was not as rapid as that described for Parelaphostrongylus spp.

The establishment of parasites is frequently reduced at high inocula. Dhar and Sharma (1978) reported that the smallest inoculum of Dictyocaulus filaria in domestic lambs had the highest percentage establishment of adults in the lungs. Similar findings were reported for experimental infections of Fasciola hepatica in domestic sheep (Boray, 1967) where it was shown that heavier infections resulted in a significantly lower percentage yield of adults than the lighter infections. These studies provide evidence that crowding may have an appreciable influence on the number of parasites that establish. The establishment of A. cantonensis in the present study, however, increased as the infective inoculum increased (Table II), suggesting that worm establishment was not influenced by crowding.

Ninety-six percent of the worms that established were found in the pulmonary arteries whereas the other 4% were found in the right ventricle of the heart. Sex ratios in these sites were not recorded; however, the overall ratio of males to females was approximately 1:1 for all groups except Group 1 (Table II). This deviation may be attributed to the small sample size (0-4 worms). The 1:1 ratio in the

present study is supported by the work of Lee (1967). He recorded 42.8% males and 57.2% females of 2500 worms found at necropsy. Sex ratios were shown to vary from 1:1 to 3:1 in the pulmonary arteries and heart, respectively.

Initially four levels of inocula were used, however two distinct groups were evident. No significant differences were found in rats that received low inocula (5 and 10 larvae) and rats that received high inocula (20 and 40 larvae); however, there were significant differences in larval production between the low and high groups ($F = 11.15$; $p < 0.0001$; Fig. 2). Larval production in the present study was apparently governed by the number of females present in the pulmonary arteries. Significantly more females were present in the pulmonary arteries of rats that received high inocula than in rats that received low inocula. Rats infected with 5 and 10 larvae yielded an average of 2 and 2.4 females at necropsy, respectively, which was significantly lower than rats infected with 20 and 40 larvae which harbored an average of 7 and 14.8 females, respectively ($t = 4.01$; $p < 0.001$). Although significantly more females established in Group 4 and in Group 3 ($t = 2.41$; $p < 0.05$), larval output between these groups was virtually the same.

The factors governing parasite fecundity are complex. There are several possibilities that may explain differences between and among the high and low inoculum groups identified in this study. First, the crowding effect may reduce parasite fecundity. Second, competition may result

in a reduced rate of egg production. Finally, overcrowding may impair egg survival and the escape of larvae from the lung parenchyma.

The size of individual parasites may decrease in proportion to the parasite burden (Kennedy, 1975). This has been commonly referred to in the parasitological literature as the crowding effect (Chandler, 1939). A size differential due to the intensity of infection was evident for Angiostrongylus costaricensis in naturally and experimentally infected Sigmodon hispidus by Morera (1973). At higher densities, competition for some limiting resource increases, and the growth of individual parasites declines. At low densities, however, competition for resources does not occur, and maximal size is attained. The limiting resource in many parasitic populations is commonly a source of carbohydrate (Roberts, 1961).

Stromberg and Crites (1975) demonstrated that a reduction in body volume results in a reduction of fecundity in Camallanus oxycephalus (Nematoda: Camallanidae). These authors estimated the number of larvae produced per female and found that the number of larvae is a function of female body volume. Larval deposition in Camallanus oxycephalus is synchronous, and larval release occurs at a single moment when the uterus prolapses (Stromberg and Crites, 1975). This differs from A. cantonensis where egg release is a continuous process.

Regression analysis of body volume as related to

population density revealed no relationship for female A. cantonensis in the present study. The absence of a negative relationship between population size and individual worm size indicates that the crowding effect is not a factor. Male angiostrongylids exhibited a significant positive relationship between body volume and intensity of infection. Reasons for this increase are unknown.

At high population densities, intraspecific competition for a limiting resource may result in a reduced rate of egg production (Kennedy, 1975). A high density of adult worms is an unfavorable component of a parasite's environment (Schad, 1977) and consequently egg production is reduced to relieve the environmental strain. This could possibly explain the reduced larval output observed in Group 4 (40 L3).

Shorb (1933), Hunninen (1935), Weinmann (1958) and Heyneman (1963) referred to an inverse relationship between the numbers of Hymenolepis nana (Cestoda: Hymenolepididae) in a given host and their mean size. Chandler (1939) further reported that one of the consequences of crowding in tapeworms is a progressive reduction in individual egg production. Ghazal and Avery (1974) have since demonstrated that crowding in H. nana populations in mice decreases the length, weight, number of proglottids, and number of eggs produced per proglottid. The crowding effect results in competition for nutrients which reduces the growth rate of individual worms, and fecundity (egg production per worms) declines.

Fecundity was defined in the present study as the number of larvae released per gram of feces per female. The crowding effect, as reflected in reduced size of adult female A. cantonensis, is not operational (see above). As a result, regression analysis of fecundity on population size failed to demonstrate any relationship between these variables (Fig. 4).

The final possibility for reduced fecundity may lie in the failure of eggs to develop and/or larvae to escape the lung parenchyma. Although quantitative estimates of lung pathology were not made in the present study, rats that received 20 and 40 infective larvae had visible lesions in the lung. Lungs were nearly three times their normal size and filled with ova. The tissue was pale and hard, and was nearly completely replaced by fibrous tissue. Granuloma-like nodules were evident in some lobes similar to those described by MacKerras and Sandars (1955) and Nishimura (1966).

The lungs of animals infected with 5 and 10 larvae did not demonstrate severe pulmonary pathology. The lungs of these animals were occassionally discolored, only slightly enlarged, and mottled in appearance.

Pathological changes resulting from the heavy infection of rats with A. cantonensis may render the lung tissue unsuitable as a habitat for developing ova (Kennedy, 1976). The diseased lung, therefore, may adversely affect the developing eggs and impair egg survival. As a result, the reduced rate of egg survival and hence, the reduced number

of L1 that appear in the feces could account for the reduced fecundity observed in Group 4 in the present study. Larvae that do survive and escape into the lung parenchyma may be unable to escape the diseased tissue, and therefore will be unable to complete their migration.

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Vita

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Grace is presently teaching eighth grade Science, ninth grade Algebra, tenth grade Biology, and 11th grade Chemistry at the Tandem School in Charlottesville, Virginia. She plans to pursue a Ph.D. degree at Washington University in St. Louis, Missouri where she and her husband will reside in the Fall, 1983.