COMPARISON BETWEEN MIGRATORY BEHAVIOR OF *TOXOCARA CANIS* AND *BAYLISASCARIS PROCYONIS* LARVAE IN ORALLY AND PARENTERALLY INFECTED MICE

by

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DEDICATION

To my father, to all fathers and mothers.

INTRODUCTION AND LITERATURE REVIEW

A. TOXOCARA CANIS (T. canis)

T. canis (Werner, 1782), the common round worm of the dog, inhabits the small intestine of the dog, fox and many other species of Canidae. The male can reach up to 10 cm long, females up to 18 cm. Eggs are with thick, finely pitted shells, measuring about 90 x 75 µ.

Infection with this parasite is considered to be of high incidence and wide geographical distribution. T. canis is considered to be, a helminth normally maturing in animals but it can migrate in human tissues, without necessarily undergoing further development. It gains access to humans, especially children between 1-4 years of age, due to their habit of eating dirt or soil contaminated with the embryonated T. canis eggs (Beaver 1959 and Beaver et al, 1952). The eggs are considered to be resistant and they can stay alive for many years in the soil because of their extra thick outer layer. When in humans, T. canis causes, visceral larva migrans (VLM): which is the migration of larvae in somatic tissues e.g. liver, lung, heart, eye and central nervous system (CNS). Nichols (1956) described a diagnostic key for the infective second stage larva of T. canis.

Beaver (1952) was the first to show a direct association between infection with T. canis and VLM. Larvae also may act as a vehicle of secondary bacterial or viral infection (Woodruff, 1970, 1975). After hatching in the small intestine, larvae migrate to liver and lung and other distant organs (Sprent, 1958). They may remain alive in tissues for long time without further development.

The life cycle varies, depending on age and sex of the dog host (Sprent 1958, and Sprent and English, 1958). Infective eggs develop in soil and molt once within the egg to become the infective second stage larva (L_2). When ingested by the adult bitch, they hatch in the small intestine, penetrate through the intestinal wall and migrate to different tissues; liver, lung, kidneys and brain with no larvae returning to the intestine or stomach. This is called "somatic migration". The larvae stay as L_2 in these tissues, viable for months or years. Larvae then migrate to fetal liver and lung, through the placenta in the pregnant bitch. They have their first parasitic molt in the fetal lung to become L_3. When L_3 larvae are found in the lung of the puppy at birth, they try "Tracheal migration", reaching the intestine and having the 2nd parasitic molt to become L_4 at about 3 days of age. The last molt occurs in the intestine to produce adult worms. After three weeks egg production occurs. The dam, at this stage, is likely to ingest the feces of the puppy and will excrete eggs in her feces which will ultimately develop to infectivity in the environment. Sprent (1961) showed that adult females can acquire intestinal infection by ingesting larvae excreted in the feces of their puppies as a result of her habit of consuming the feces of her pups while they are suckling. This infested feces contains numbers of advanced stage larvae (L_3) which are able to develop into adult worms in the bitch's intestine, such infection is usually short lived.

If puppies up to 3 weeks of age ingest the infective eggs they develop adult worms in the small intestine i.e. tracheal migration, occurs. But if puppies are 5 weeks of age or over, such ingestion results in somatic migration mainly (Sprent 1958 and Webster 1958). In either

prenatal or early postnatal infection adult worms exist in the small intestine for at least six months, thereafter the incidence is progressively reduced. Male dogs are more susptible to intestinal infection than females (Taylor, 1964).

Behavior of orally inoculated infective eggs in their normal host and in numbers of abnormal experimental hosts have been studied. Experimental hosts that have been used include: mice (Lee 1960, Smith and Beaver, 1953, Oshima 1961, Beaver 1962); rabbits (Beaver 1962, Fernando 1968); rats (Beaver 1962); guinea pigs (Beaver 1962); pigs (Done et al. 1960); sheep (Schaeffle 1960); calves (Fitzgerald and Mansfield 1970); baboons (Aljeboori and Ivey 1970 and Aljeboori et al. 1970); chickens (Odoshi and Usui 1968) and earthworms (Okoshi and Usui, 1968). Toxocara canis eggs have been used by different workers to show the ultimate fate of the larvae in the host. Variable results and conclusions have been presented with no satisfactory explanation for low percent larval recovery from tissue digestion.

None of the workers have studied the fate of injected L_2 larvae of T. canis intravenously. It has been done with other ascarids using normal and abnormal hosts (Arean and Crandall, 1962) and (Melendez and Lindquist 1979).

B. BAYLISASCARIS PROCYONIS (B. procyonis)

B. procyonis, is an ascarid nematode, found in small intestine of the raccoon Procyon lotor. It was first discovered by Olsen and Fenstermacher (1938). Adult males are about 9 cm and adult females 20 cm in length. The thick shelled eggs are 65 µ wide by 79 µ long (Fig. 1).

Tiner, (1952) was able to differentiate it from the ascarid which occurs in the skunk, previously they were considered to be the same worm.

It has been shown by Tiner (1951, 1953 a & b) that this particular ascarid out of many other ascarids can cause fatalities in different rodents. Death has been attributed to the presence of the larvae in the brain which cause central nervous system symptoms.

The life cycle in the normal host, raccoon, has not been worked out. Tiner 1953a fed B. procyonis eggs to different rodents; grey squirrles, hamsters, cotton rats and guinea pigs. He found that this ascarid migrated to the brain and caused fatalities among those rodents. He also noticed, the encystment of the larvae in the thoracic viscera. After the work by Tiner, there was nothing done concerning the migration and distribution of larvae of B. procyonis in mice.

The objectives of this study were to compare the fate and distribution of larvae in parenteral and oral infections using T. canis and B. procyonis. These infections were followed at varying time intervals.

MATERIALS AND METHODS

All tubes, dishes, rubber tubing, syringes, needles and every laboratory ware that might come in contact with the egg suspensions, were coated with suspension of Siliclad*. The reason this was done because it is known that ascarid eggs are very sticky due to their outer protineous membrane and tend to adhere to surfaces very readily.

A. INOCULUM PREPARATION

1. EGGS

a. TOXOCARA CANIS

Eggs of T. canis were collected from feces of infected dogs and puppies. The positive feces was first passed through an 80 mesh screen into a graduate cylinder with cold tap water. The graduate cylinder containing the fecal suspension was left for 4 hours in the refrigerator for the eggs to settle. The sediment was then washed with cold tap water and left in the refrigerator for another 4 hours. This was repeated several times until there was a clean supernatant. The eggs were floated out of the sediment using zinc sulphate solution (sp. g. 1.12) and centrifugation at 160 g for 5 min. For embryonation the eggs were left in petri dishes containing 0.5% formalin at a depth of ¼" for 3-4 weeks at room temperature, with frequent agitation. After that time the eggs were ready for oral infection or hatching.

b. BAYLISASCARIS PROCYONIS EGGS

Eggs of B. procyonis were collected from adult female worms from the small intestine of infected raccoons brought to the laboratory by

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*Clay Adams

raccoon hunters. Females usually were alive at the time of arrival to the laboratory. They were left in physiological saline (0.85% NaCl) in 37°C incubator for 3-4 days. The saline was changed once a day and the eggs were concentrated by centrifugation for 5 minutes. When females did not lay eggs, the vagina and part of the uterus about one centimeter in length were dissected out from the rest of the worm. Eggs were sequeezed out of these structures and left for embryonation as previously described for T. canis eggs.

When ready to use, the eggs were washed out of the formalin by centrifugation several times with distilled water and then used for infection.

2. LARVAE

All tubes and dishes were sterile when starting this technique. Hatching of eggs of both T. canis and B. procyonis, was performed using method of Arean and Crandall (1962) with slight modification. The eggs were suspended overnight at room temperature in solution of equal parts (20 ml) of 2% sodium hydroxide and 2% sodium hypochlorite (using commercial chlorox). This treatment of the eggs completely removed the outer two layers, leaving only a thin vetelline membrane.

The next morning, the egg suspension was washed several times by centrifugation for 5 minutes at 160 g with sterile physiological saline, until no odor of sodium hypochlorite was apparent. The suspension of eggs in sterile saline was agitated with magnetic stirrer for 3-4 hours at room temperature. The eggs were then centrifuged at 800 g for several times until a high percentage of hatching was noticed after taking samples and examining them under the microscope. Larvae were counted and

were given to the mice on the same day of hatching.

3. COUNTING

The egg or larval suspensions were mixed thoroughly, 0.5 ml sample was taken with a pipette and divided into ten 0.05 ml aliquots, each on a slide. This was done very fast to eliminate the possibility of eggs settling down more in the first aliquots. All of the ten aliquots were counted to learn whether the eggs were equally distributed to all of the 0.05 ml aliquots. This process was repeated three times since this step is very important in getting the right number of eggs for each dose. The same procedure was done with the larvae except the 0.05 ml aliquots of larval suspensions were killed first with Lugol's iodine solution before counting to stop the active movement of the larvae particularly that of *T. canis*.

B. EXPERIMENTAL MICE

Mice were chosen as the experimental animal in this study, because of their small organs which allowed total and careful accurate count, (Sprent 1952).

Six to eight week old mice were used. They were examined before infection for intestinal parasites by using the zinc sulphate fecal floatation method. Some mice were found to harbour two kinds of oxyurids (*Syphacia* and *Aspiculuris*), others harboured only one of these. Such mice were excluded from the experiment.

1. ALLOCATION AND EXPERIMENTAL DESIGN

A total of 148 mice was used in a total of 8 experiments. Four experiments had 18 mice each and the other four had 19 mice each. Two routes of infection were used; oral with eggs and intravenous (I.V.) by the tail vein, with larvae. Two levels of dosage were used; 500 and 3000. Allocation of mice is shown in Table I.

Table I - Distribution of the 148 mice to eight different experiments

	Experi-ment	Level of dose	# of mice	Route of Infection
I. *T. canis*	1	500 eggs	18	Oral
	2	3000 eggs	18	Oral
	3	500 larvae	18	I.V.
	4	3000 larvae	18	I.V.
II. *B. procyonis*	5	500 eggs	19	Oral
	6	3000 eggs	19	Oral
	7	500 larvae	19	I.V.
	8	3000 larvae	19	I.V.

2. INOCULATION PROCEDURE

a. ORAL INOCULATION WITH EGGS

Mice were intubated to their stomach with the specific dose level of eggs, by using 1 mm diameter plastic tube connected to 22 gauge, 1" long needle attached to a tuberculin syringe. When dosing with eggs, the loaded syringe was held perpendicular with the needle pointing down, so that the maximum number of eggs was discharged into the stomach. The system was flushed by 0.3 ml water to rinse through remaining eggs in the syringe

and tubing. The inoculum was suspended in an amount of water not exceeding 0.5 ml.

After oral inoculation with eggs, mice were kept individually in cages, for 24 hrs and all feces were collected and examined for eggs or larvae which might have passed through with the feces. Zinc sulphate floatation technique was used to detect such eggs and larvae.

b. INTRAVENOUS INJECTION WITH LARVAE

Hatched larvae were given on the same day to the mice by intravenous injection in the tail vein. A 27 gauge, $\frac{1}{2}$" long needle attached to 1 ml tuberculin syringe was used. Mice were anaesthetized lightly with chloroform, then put in an acrylic restraining cage for intravenous injection of the larval suspension in an amount of physiological saline not exceeding 0.5 ml.

c. FOLLOWING MIGRATION OF LARVAE IN MOUSE TISSUES

Two doses of eggs and larvae; 500 and 3000 were used. Mice were sacrified by using choloroform, one per day from 1-9 days after infection and thereafter on the 11th day, then 3 days apart until the 26th day, and at the 38th, 50th and 60th day after infection.

Each time a mouse was sacrified, the different organs (Brain, lung, liver, spleen, kidney, heart, stomach and intestines and carcass) were excised and digested with pepsin or trypsin, depending on the time interval of infection and the organ being digested. Each digested organ suspension was washed from digesting fluid upon completion and run through a Baermann funnel for 4-6 hrs to retrieve any migrating larvae. The soft tissue of brain was not digested, but slices were squashed between two glass slides

to look for larvae.

Digestion of different organs except the carcass (bones + musculature), was done following Sprent's method (1952) for digestion, using trypsin as digestive fluid. Skin, tail and feet were discarded. Carcass up to 8 days after infection were digested using trypsin, while those after 8 days were digested using pepsin because Sprent (1952) noticed that larvae less than 8 days old were immobilized by peptic digestion and were carried to the top and poured off with supernatant. While trypsin did not cause immobilization of such larvae. Using pepsin 8 days after infection was to release any encapsulated larvae in the carcass that were not susceptible to trypsin digestion.

1. DIGESTION OF THE CARCASS UP TO EIGHT DAYS AFTER INFECTION

The carcass was first cut to small pieces, then ground in a Waring blender for 60 sec. with 50 ml of physiological saline (PSS). The solution was mixed with 50 ml PSS containing one gram of trypsin. The pH was adjusted to 7 with 0.1 N sodium hydroxide. The solution was left at 37°C for 4-8 hrs until a fine sediment was noticed at the bottom. It was stirred every hour or so. The suspension was washed from the digestive fluid with PSS using 160 g centrifugation. It was then run through a Baermann funnel at room temperature for 4-6 hours. The migrating larvae were found at the bottom of the centrifuge tube attached to the Baermann funnel.

2. DIGESTION OF THE CARCASS AFTER EIGHT DAYS OF INFECTION

The same procedure was followed except pepsin was used instead of trypsin and the pH was adjusted to 1 with HCl. The incubation at 37°C

using pepsin was shorter than that of trypsin.

3. DIGESTION OF LIVER, LUNGS, KIDNEYS, HEART AND SPLEEN

Each organ was cut separately into small pieces and ground in 25 ml PSS for 45 sec. in the blender. Each organ suspension was added to 5 ml PSS containing 0.05 gm of trypsin. The pH was adjusted to 7 with 0.1 N sodium hydroxide and the solution was incubated at 37°C for 3-4 hrs with frequent stirring. Each suspension was washed with PSS, centrifuged at 160 g and run through the Baermann at room temperature for 4-6 hours. Larvae accumulated at the bottom of the centrifuge tube attached to the Baermann funnel.

4. DIGESTION OF STOMACH AND INTESTINE

The same procedure was used as in part 3 but the grinding time was increased to 60 seconds. The counting of retrieved larvae was performed by centrifuge washing the sediment with PSS, aspirating the supernate and resuspending the sediment with 5 ml PSS. This was emptied into a small petri dish and larvae counted under a stereoscope.

Measurements of larvae were performed on heat killed larvae obtained after digestion. The percent larval recovery was calculated as below:

Small pieces from each organ except the carcass were kept in 10% formalin for later sectioning. Serial sections were performed at 6 μ thickness and stained with Haematoxylin and Eosin (H & E).

$$100 \times \frac{\text{Total number of larvae retrieved from various tissues digested}}{\text{Number of larvae or eggs inoculated}}$$

RESULTS

A. T. canis

1. ORAL ROUTE

a. 500 EGGS T. canis (Appendix - Table 1)

Twenty four hours after dosing, most of the larvae were found in the liver with some being in the intestine. The number of larvae in the liver decreased until day 8 after which there were no larvae recovered from the liver (Appendix - Table 1). There were no gross lesions on the liver on any day after infection but larvae were seen on histologic sections (Fig. 2).

The lung had larvae on day 2 (Fig. 3) until day 8 after which larvae were not recovered from the lung. Many piticheal hemorrhages on the lungs that were 1-2 mm in diameter, appeared on day two. They decreased in number until day 26 when no more spots were seen on the lungs. Visibly infected lungs were larger in size than the normal lungs.

The kidneys had some larvae from day 2 until day 8 after infection. There were numerous, 1-2 mm diameter white lesions on the surface of both kidneys. These persisted until day fifty. Infected kidneys were hypertrophied.

A few larvae were recovered from the heart on day 2 to day 4, day 6 to day 9 and again on day fifty. There were small numbers of larvae found in the intestine on days 1, 2 and three.

Larvae were initially recovered from the brain on day 3, and continued to be present until day 60 (Fig. 4). Most of the larvae harvested from tissue digestion after day 8 were confined to the brain. Big haemorrhagic spots were on the surface of the cerebral hemisphers. They were 3-4 mm

Fig. 1. Embryonated eggs of *B. procyonis*

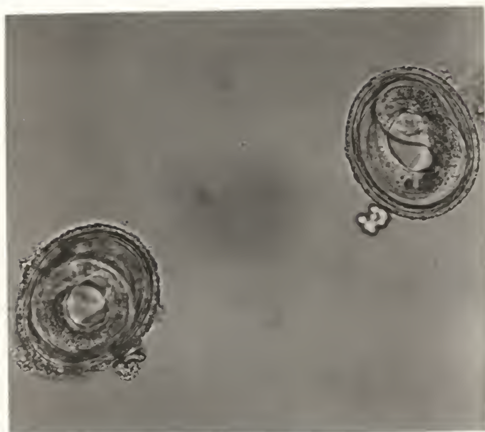


Fig. 1

Fig. 2. *T. canis* larva in a liver section, two days after oral dosing with 500 eggs.

Fig. 3. Coild larva of *T. canis* in the lung section two days after oral infection with 500 eggs.

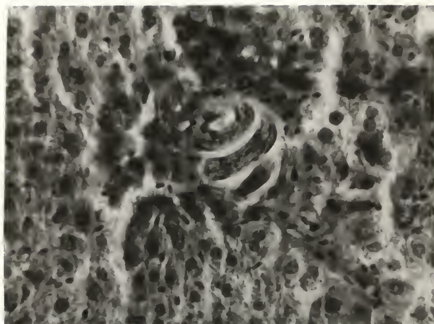


Fig. 2

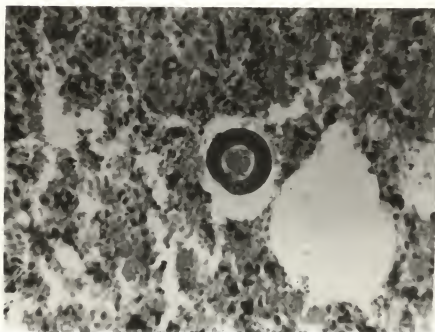


Fig. 3

in diameter, appearing on day six. These spots decreased in size until disappearance on day fourteen.

Larvae were found in the carcass on day 2 through day nine. No lesions were seen on any part of the carcass. Some small hemorrhagic foci were present on the internal surface of the skin but no larvae were demonstrated.

There was no appreciable increase in length or width of larvae recovered from the digestion of organs even up to 60 days after infection (Appendix - Table 9).

None of the infected mice showed any signs of central nervous system (CNS) involvement as a result of residence of larvae in the brain from day 3 until the 60th day. There were no deaths among the infected mice through 60 days of infection. Per cent larval recovery was the highest (64.2%) on the day 1 but it started to decrease from then on. Loss of eggs that failed to hatch and larvae that failed to penetrate the intestine after dosing were not examined in this dose, so per cent recovery represents only larvae recovered from organ digestions.

b. 3000 EGGS T. canis (Appendix - Table 2)

Among all organs the intestine had the largest per cent larval recovery (78.8%) in day 1 after infection. This number decreased until day 9 when only four larvae were recovered (Fig. 5). The larvae re-appeared on day 14, 23, 38 and 60 (Plate 1-I).

Larvae were recovered from the liver on day 1 and larval recovery increased on day 2 and peaked at day three. About 90.80% of the total larval recovery on day 3 was in the liver. After day 3 the larval number started to decrease until no larvae were found on days 50 and 60

Fig. 4. Brain section, eleven days after oral infection with 500 eggs of *T. canis*.

Fig. 5. Intestinal section on the 1st day after oral dosing with 3000 eggs *T. canis*, the larva has left the intestinal lumen and penetrated to the outer muscular layer.

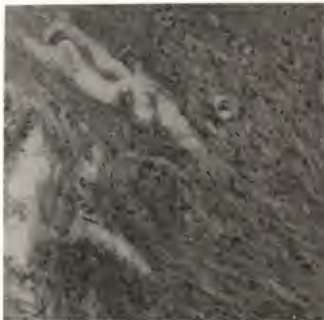


Fig. 4

Fig. 5

(Plate 1-R). The white spots on the liver appeared on day 2, 3 and 4 after infection.

The peak of larval recovery for the lungs occured on day 4 after which there was a decrease in larval number up to day eight. After day 8 larvae were found irregularly in the lungs (Plate 1-G). Lesions appeared on day 3 and increased on day four. On days 5, 6 and 7 the lungs were so congested that they had the appearance of the liver. Lesions were still present until day seventeen.

In the kidneys, the largest number of larval recovery was on day 3 (Fig. 6) and the larvae appeared in the kidneys in variable numbers up to day 60 (Plate 1-K). The white spots were seen first on the kidneys on day 5 and persisted through day sixty.

Irregular small numbers of larvae were found in the heart and they continued to be seen until day 60 (Plate 1-H).

Rare larvae were seen in the brain as early as day 2, they increased in number after that day and were found until day 60 (Plate 1-B). Two 3-4 mm diameter hemorrhagic spots were seen on both of the cerebral halves on day five. These lesions sequentially decreased in diameter and disappeared by day seventeen.

Larvae appeared in the carcass on day 3 and persisted until day 50 (Plate 1-C).

At this dose level, there was no appreciable increase in length or width of recovered larvae from any organ during the period of infection (Appendix - Table 10) and (Plate 2: B, C, G, H, I, K, R).

Dosing even with 3000 eggs of _T. canis_ did not cause any CNS symptoms or death even after 50-60 days after infection. The highest percentage

Fig. 6. Kidney section shows larva of *T. canis*, six days after dosing orally with 3000 eggs.

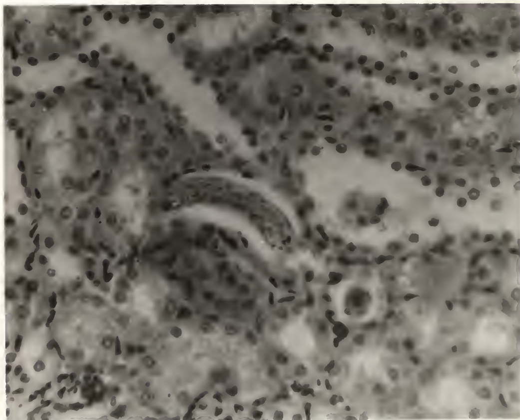
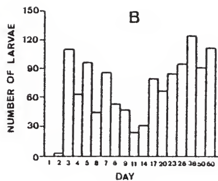


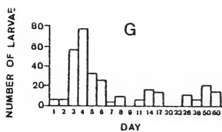
Fig. 6

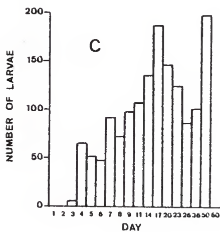
Plate 1 - Distribution of recovered larvae of _T_. _canis_ after digestion of different organs at different time intervals

B = Brain, C = Carcass, G = Lungs, H = Heart, I* = Intestine, K = Kidneys, R* = Liver

*Number of recovered larvae was divided by 10 and then plotted against days after infection.







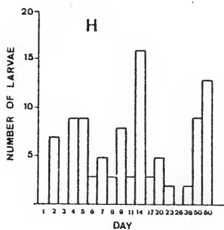
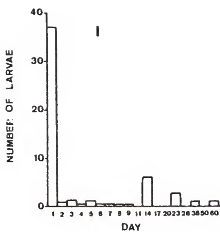
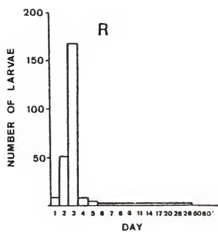


Plate 1: B, C, G, H





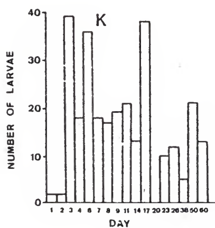


Plate 1: I, K, R

Plate 2 - Lengths (in μ) of recovered larvae of _T_. _canis_ from digestion of different organs, plotted against the time in days.

B = Brain, C = Carcass, G = Lungs, H = Heart, I = Intestine, K = Kidneys, R = Liver

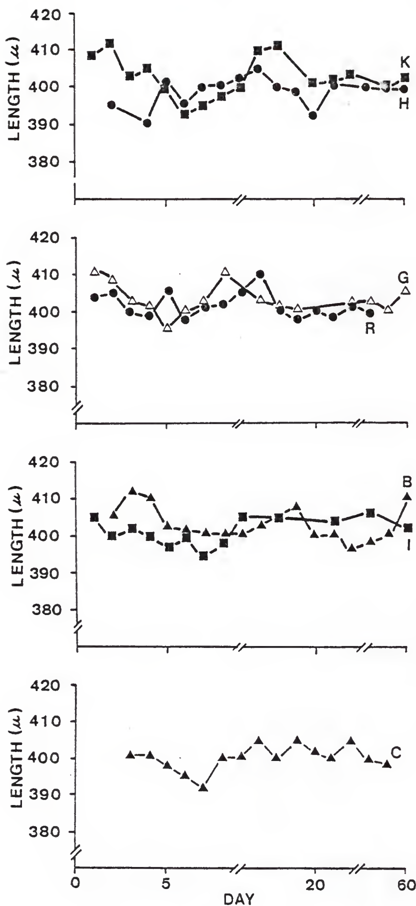


Plate 2: B, C, G, H, I, K, R

of larval recovery was on day three. After day 8 most larvae were found in the brain, carcass and sometimes in kidneys and intestine. They continued to reside in these organs till the end of the experiment. No cysts were found in the carcass. That was true with the previous dose of 500 eggs of T. canis.

2. PARENTERAL ROUTE

a. 500 LARVAE T. canis (Appendix - Table 3 & 11)

Large numbers of larvae, given parenterally, were recovered from the lung on day 1 after infection. The number decreased on successive days until day 7 on which the greatest number of larvae were recovered and after which few numbers were present in the lung until the end of the experiment. Piticheal Hemorrhages were present on the lungs on the 1st day. Lungs were congested on days four. The lesions became lighter in color on progressive days until day 20 when no more were seen on the lungs or on any other organ.

Larvae were found in the liver on day 1, 3 and until day 9, when t they disappeared. They reappeared on day 11 but in fewer numbers. Larvae were there until the 60th day. No gross lesions were seen on any day.

Larvae were found in the kidneys on day 1 after infection. They increased in numbers on successive days until day 8 when their numbers declined. The number increased on day 26 until the 60th day. Day 5 showed the presence of some white spots on the kidneys, but they receded and disappeared by day fourteen.

Some larvae were recovered in small numbers from the heart. They appeared on day 2, they were not found on day 9, and after the 26the day. The same pattern was noticed in the intestine.

Larvae migrated to the brain on day 1 and to the carcass on 2nd day. They persisted in these organs until the 60th day. Hemorrhagic spots appeared on the brain surface on day 2, but no more were seen after day five. After day 8, the majority of larvae were found in the brain, carcass and kidneys. Occassionally some larvae were found in the spleen. There was no increase in length or width of the recovered larvae or any CNS involvement.

b. 3000 LARVAE T. canis (Appendix - Table 4 & 12)

Many larvae were found in the liver until day 14 and the number decreased steadily to day sixty. Lesions were not seen on this organ on any day.

The largest number of larvae were present in the lungs on day 1 after infection. There was a decrease until day 8 and rare larvae were seen until the 60th day. Same lesions appeared on the lung on the day 1 and increased in number on day 2, from then on they decreased until day 20 when they were not found on the lungs or other organs.

In the kidneys, larvae appeared in large numbers on day 3 and persisted until the 60th day. White lesions were present on the kidneys from day 4 to the twentieth day. In the heart, spleen and intestine larvae appeared on day 2 and remained in the heart until the day 50, after which they were not seen. They were sporadically found in the intestine and spleen.

Larvae were in the brain and carcass by day 2 and remained until day 60, they were found in large numbers. Hemorrhagic lesions were seen first in the brain on day 2 and persisted until the 9th day.

The average per cent larval recovery using the parenteral route of infection was higher than using oral route (29.3% and 19.8% respectively) of infection with T. canis.

B. BAYLISASCARIS PROCYONIS

1. ORAL ROUTE

a. 500 EGGS (Appendix - Table 5)

In the liver larvae were seen on day 2 and 3 with no gross lesions on this organ. The lung had larvae on day 1 and 2 although few lesions were seen on the lungs from day 2 till the 9th day. Only two larvae were found in the kidneys on day 6 with no lesions.

In the intestine, the larvae were recovered on days, 1, 2 and 3 and again on days 15, 16 and 17th day. Larvae were recovered from the brain from day 3 to day 7 (Fig. 7). Gross lesions appeared on the brain on day 3 only. In the carcass larvae were found from the day 4 through the seventeenth. No larvae were found in the spleen.

Superficial white cysts appeared on the surface of the heart. These cysts measured 1-1.5 mm in diameter, and first appeared on day 8 and continued until the end of the experiment (Fig. 8). Microscopic examination of these cysts revealed one and sometimes two living larvae in each cyst. Before day 8 there were some free larvae found in the heart. Similar cysts appeared on the diaphragm on the surface facing the peritoneal cavity. These cysts appeared on day 4 and they were found on each successive day of the experiment.

Twelve days after infection signs of a CNS involvement appeared on most of the infected mice. On day 13 all infected mice had CNS signs.

Fig. 7. *B. procyonis* in brain section, on the 15th day after oral infection with 500 eggs.

Fig. 8. Cross section of a heart cyst produced fifteen days after dosing with 500 eggs *B. procyonis*.

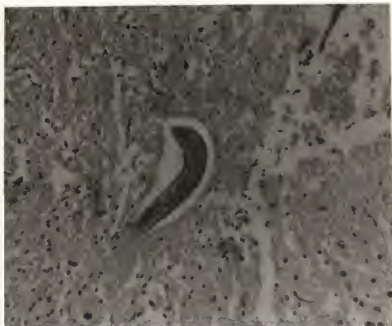


Fig. 7

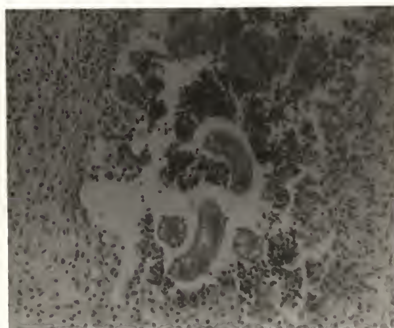


Fig. 8

The mice were easily disturbed by a slight movement of their cage. They went around the cage undirectionally or jumped high enough to escape from the cage. By day 14 they started a circular movement and by the next day they were recumbant. They moved if touched but reclined on one side, although both sides were responsive. They stayed moribund through days 13, 16 and 17, failing to eat or drink after day 12 of infection. Three mice died on day 15 and two more on day 16 and the last two died on day 17 after infection.

Loss of eggs and larvae that failed penetration after dosing was less in numbers relative to that of T. canis in the oral infections (Appendix - Table 5).

The larvae in the heart, intestine, brain and diaphragm grew in length and width, although they were surrounded by cysts in the heart and diaphragm. Larvae did not grow much in the carcass compared to the previous organs. The maximum measurements were 1290 x 55 μ in the brain on day 17, but in the carcass they grew only to a maximum of 333 x 23 μ (Appendix - Table 13). On day 17 the brain was very soft and friable, the 8 larvae in that brain averaged 1290 x 55 μ.

b. 3000 EGGS B. procyonis (Appendix - Table 6)

This experiment lasted 14 days, although it was planned for 60 days. Infected mice began to exhibit nervous signs earlier than the previous dose (500 eggs of B. procyonis) and they all died before day 15 following infection.

Larvae appeared on day 1 in the liver, increased in number on day 2 and declined until day 6 when they no longer could be found

(Plate 4-R). No lesions were seen on the liver. The lungs had the greatest number on day 1, the number decreased until day 5 when larvae disappeared (Plate 4-G). Lesions were present on lungs from day 1 until the 7th day.

Larvae appeared in the kidney on day 3 but no gross lesions were noticed (Plate 4-K). In the heart, free larvae appeared on day 4, and were encapsulated on day 9 (Plate 4-H). Cysts appeared on the diaphragm on day 5 until the end of the experiment (Plate 4-D).

Larvae appeared in the brain and carcass on day 4 and persisted, until the final day (Plate 4-C, B). Hemorrhagic lesions were noticed on the brain on day 4 and the 5th day.

The nervous signs began on day 8 after infection except in one mouse that had the signs on the next day. Infected mice stopped eating and drinking when the signs appeared. These signs were not gradual as they were with the 500 egg dose. They were more severe and developed rapidly after they appeared on day 8, on the next day all mice were lying on one side and moribund until death. Two mice died on days 9, 11 and 12, five mice died on the 14th day.

Larvae increased in length in the diaphragm, heart, intestine and brain (Plate 3). They reached their maximum length in the diaphragm On day 11 after infection (Appendix - Table 14).

Loss of larvae and eggs through feces after dosing was small (Appendix - Table 6).

2. PARENTERAL ROUTE

a. 500 LARVAE B. procyonis

No larvae or lesions were found in the liver or kidneys of infected mice. In the lungs the greatest number of larvae were found on day 1 and decreased until day 5 when no larvae were recovered. The larvae reappeared from day 6 to the 11th day. Hemorrhagic lesions were present on the lungs from day 1 to day nine.

Some larvae were found free in the heart on day three. Cysts appeared on the heart (Fig. 9 & 10) and diaphragm on the 7th day. They were found until the end of this experiment.

Larvae appeared on days 4, 7 and 8 after infection in the intestine. In the carcass larvae were present on days 3 and 11 only. Larvae were present in the brain on day 5 and persisted until death. Small hemorrhagic spots were noticed on the brain on days 5, 6 and 7 after infection. No larvae were found in the spleen.

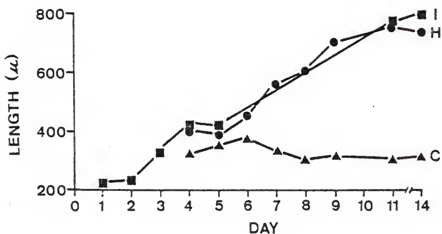
Signs of CNS disease appeared on day 7 after infection in ten of twelve mice. On day 8 the rest of the mice showed the CNS signs. On day 7 one mouse died, two on day 8, three on days 9 and 10 and four on the eleventh. The signs were not gradual.

Although larvae were found in the lungs until the last day (except day 5) they did not grow in length or width very much even to the last day of the experiment. Larvae increased in length and width in the heart, brain and diaphragm. They reached a maximum size of 1075 x 45μ in the heart on the 11th day. Larvae did not grow as much in the carcass (Appendix - Table 15).

Plate 3 - Distribution of recovered larvae of *B. procyonis* after tissue digestion and Baerman use in different organs at different time intervals.

B = Brain, C - Carcass, D = Diaphragm, H = Heart

I = Intestine, K = Kidneys, R = Liver



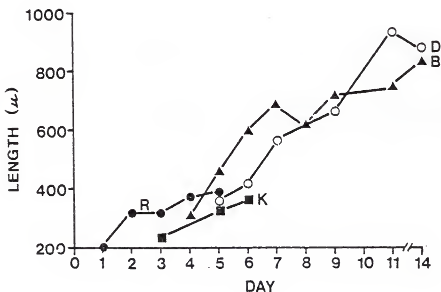
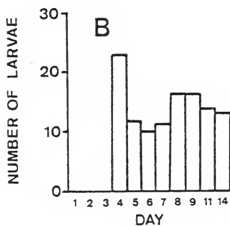


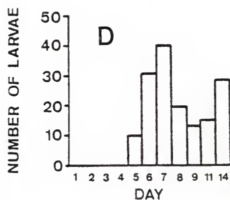
Plate 3: B, C, D, H, I, K, R

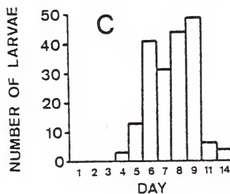
Plate 4 - Lengths (in μ) of recovered larvae of B. procyonis from digestion of different organs, plotted against the time in days.

B = Brain, C = Carcass, D = Diaphragm, G = Lungs, H = Heart, I* = Intestine, K = Kidneys, R = Liver

*Number of recovered larvae from the intestine was devided by 10.







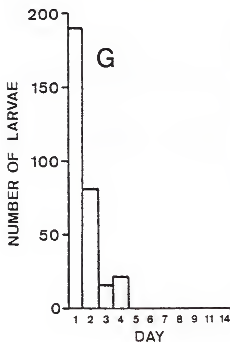
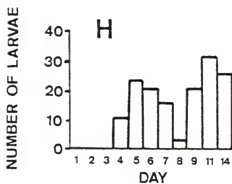
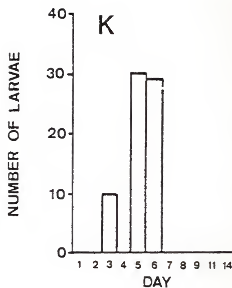
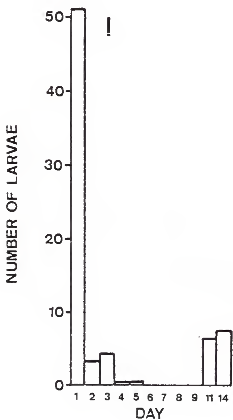


PLATE 4: B, C, D, G







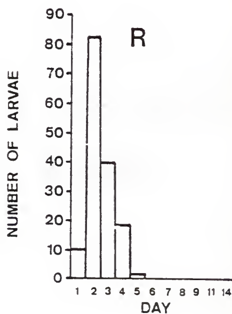


PLATE 4: H, I, K, R

b. 3000 LARVAE B. procyonis

No larvae or lesions were found in the liver or kidneys. About 96% of the total larval recovery on day 1 was in the lung and decreased in number thereafter. No larvae were found on day 4 but were recovered on day 5 and persisted until termination of the experiment. Lesions appeared on days 2 to 6 when the lungs became very congested. On day 7 the lungs were still congested lesions were fewer in number on the 8th and 9th day. No larvae were found in the spleen.

Cysts appeared on the heart on days 4, 6, 7, 8 and 9, in the diaphragm on days 8 and 9 (Figs. 11, 12, 13). Larvae were recovered from the carcass on days, 1, 3 and the 4th. In the intestine larvae appeared on days 3, 4, 6, 8 and 9 days post infection. Larvae appeared in the brain on day 5 (Fig. 14) and persisted to the end of experiment. Small irregular hemorrhagic spots were seen on the brain on days 4 and the 5th day. Brain on day 8 and 9 had no gross lesions.

On day 4 most of the mice seemed depressed and dull. On day 6 CNS signs appeared on all of the mice. One mouse died on day 7, three died on day 8 and two were moribund. On day 9 the rest died (7 mice).

The larvae grew in length and width in the diaphragm, brain, intestine and heart. They did not increase in size in the lungs or carcass (Appendix - Table 16).

Fig. 9. Pressed preparation of heart cyst shows larva of *B. procyonis*, eight days after intravenous injection of 500 larvae.

Fig. 10. Larva of *B. procyonis* measured 870X45 μ, in heart pressed tissue after intravenous injection with 500 larvae.

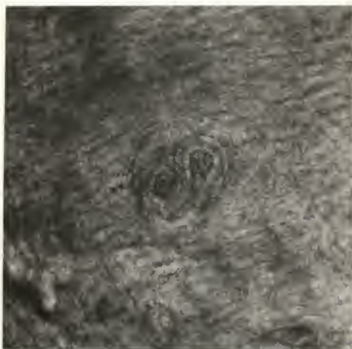


Fig. 9

Fig. 10

Fig. 11. Diaphragm section shows larva of _B. procyonis_ encysted, sixteen days post parenteral dosing with 3000 larvae.

Fig. 12. Piece of diaphragm, pressed between two slides to show the encysted larva of _B. procyonis_ after parenteral infection with 3000 larvae.

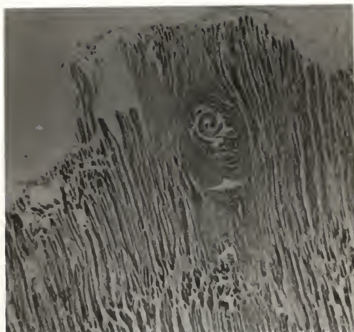


Fig. 11

Fig. 12

Fig. 13. Gross diaphragm cysts produced by B. procyonis, eight days after injection with 3000 larvae.

Fig. 14. Piece of brain, pressed between two slides to show larva of B. procyonis on the 9th day after injection of 3000 larvae. The larva is casting its luter cuticle.

Fig. 13

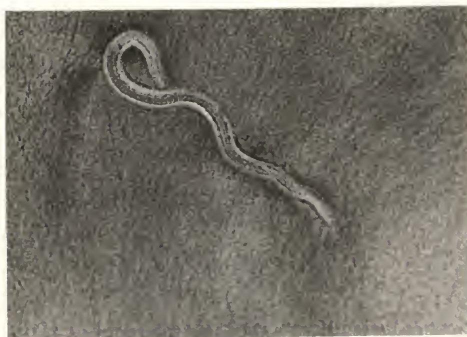


Fig. 14

DISCUSSION

Many authors have found that T. canis follows a somatic migration in abnormal hosts, as it does in human tissues (Sprent 1963 and 1958, Greve 1971 and Woodruff 1970). When T. canis eggs are given orally, part of the dose hatches within several hours (2-4) in the stomach and intestine, the larvae invade the mucosal lining of these organs but the maximum number invade that part which is 25-50 cm away from the pylorus (Chari and Subramanian 1972). Different routes for the migration of larvae from the intestine to other organs have been suggested by many workers. Histopathologic studies have shown that after penetration of the intestinal wall into the abdominal cavity, larvae may penetrate the liver directly, as there was no evidence of lesions in the neighbouring liver tissues which could be attributed to internal migration. Alternatively larvae can reach the liver through the mesentry by the portal tracts from hepatic portal system after penetration of the hepatic portal vein and then pass to the lungs (Burren 1967 and Bisseru 1969). Many of these portal tracts showed cellular infiltration which could be seen from the portal tracts through the lobules to branches of the hepatic vein. Webster (1958) suggested that few larvae reach the liver by this route. Larvae can also migrate from the hepatic vein into the inferior vena cava (Nichols 1956) or they can reach the liver via the mesentric veins and lymph nodes and lumphatic vessels. There are several reports of the recovery of ascarid larvae from the lymphatic system in many hosts. Burren (1967) recovered T. canis larvae from the lymph nodes of the mouse. Webster (1958) suggested that in the dog most T. canis larvae enter the lymph vessels after penetrating the intestinal wall.

However, Warren (1969) after feeding 25,000 eggs of T. canis to mice noted that part of the larvae were in the abdominal and thoracic cavities and in many other organs including the diaphragm after 4 days. Chari and Subramanian (1972) found some larvae in the peritoneal cavity 96 hours after infection. However Yoshida (1919) believed that Ascaris larvae migrate in the body of the mouse through direct boring of different organs. Burren (1967) found numerous lesions associated with branches of the hepatic vein and concluded that larvae leave the liver by way of the blood stream. When in the blood, the larvae were carried to the heart and hence to the lungs, which they entered by passing through the walls of the branches of the pulmonary vein. Webster (1958) and Nichols (1956) found larvae in the heart and pulmonary artery 72 hours after infection and they concluded that it is the normal path of the larvae from liver to the lungs. Larvae leaving the lungs could do so either by the pulmonary vein to the heart and circulatory system and then be distributed to either the brain and the musculature, or by ascending the trachea from the bronchioles after rupturing the alveolar capillaries and re-enter the intestine (Burren 1967, Webster 1958, Nichols 1956, and Oshima 1961). Larvae can also leave the lungs through the capsule to enter the thoracic musculature after traversing the thoracic cavity (Burren 1967). Warren (1969) found T. canis larvae in thoracic cavity washings, which supported this suggested route. Sinha and Bihar (1966), on finding larvae in whole blood, suggested that migration occurred through blood. Supporting that assumption was the presence of the cerebral hemorrhages in the early days after infection (Sprent 1955 a & b). He concluded that the size and shape of the body of the larvae controlled the kind of vessels they

entered. When they reached a vessel that approached their diameter, the larvae left that vessel. This explains the presence of the hemorrhagic spots on the surface of the cerebral hemisphere. Tiner (1953a) suggested that the chance wandering of the larvae of raccoon, badger and skunk ascarids in the lungs could lead them to a venule to the heart and finally to the arterial blood to be distributed to various organs. The same chance wandering could lead them into respiratory bronchioles and eventually swallowed down the intestine.

Based on our results, it seems likely that larvae took all of the routes suggested by workers. They can follow somatic as well as tracheal migration in the body of the mouse which suggests that larvae wander randomly until they reach the sites for which they have a predilection. This might be more true for _B. procyonis_ as these larvae, once they reach the brain, began to grow in width and length, a fact that will not allow them to return to any other tissue via the blood vessels especially those that are less in diameter than the larvae. We think it will be a very interesting research if one would use more than one radio-label for the larvae before inoculation and then follow the labels into the mouse tissue.

The ability of larvae to move seems to determine the pattern of their distribution to the tissues since we did not find larvae of _B. procyonis_ in the kidney or the liver when using the parenteral route but we did with _T. canis_. We have reasoned that, were larvae to go to the kidney or liver they would have to go against the blood flow, an ability which may be possessed by the very active larvae of _T. canis_ but not the more lethargic larvae of _B. procyonis_ or perhaps these larvae don't show

predilection to these organs. We have attributed the formation of capsules around B. procyonis but not T. canis for the same reason.

A large percentage of larvae of T. canis resided in the brain and carcass tissues in the final stages of the experiment. Their predilection to these tissues has been found by others (Olson 1962, Okoshi and Usui 1968, Beaver 1956, Oshima 1961). We have suggested that this might be due to less resistance offered by these tissues against the larvae of T. canis, since we have not been able to find encapsulated larvae in the carcass or inflammatory reactions in the brain as a result of residence of many larvae in the final stages as some workers did (Lee 1960, Okoshi and Usui 1968, Beaver 1956, Burren 1967, Sprent 1952). Beaver (1959) found that encapsulation in the carcass is slow and larvae of T. canis might stay free up to two years.

We did not see appreciable growth of T. canis larvae in any organ even up to 60 days after infection and even in the tissues for which T. canis larvae showed a predilection. This agrees with other workers who studied the migration of this nematode in the abnormal host (Nichols 1956, Tiner 1953b, Smith and Beaver 1953, Done et al. 1960). Although Hoeppli et al. (1949) found growth of T. canis and adults of T. canis in humans. Schacher (1957) and Sprent (1963) independently concluded that in both cases the species involved was Toxascaris leonina not T. canis.

In spite of residence of T. canis larvae in large numbers in the brain, we did not have any CNS signs even with the 3000 dose which agrees with the results previously reported (Burren 1969, Oshima 1961, Sprent 1952 and Olsen 1962). Sprent (1955b) noticed symptoms in one out of 23 mice infected with T. canis. Some other deaths have been attributed

to verminous pneumonia caused by the invasion of the lungs by large numbers of larvae.

The average per cent recovery of larvae for T. canis was 19.8% for the oral route and 29.3% for the parenteral route. For B. procyonis the recovery rate was 11.25% for the oral and 12.2% for the parenteral route. Other investigators had different per cent larval recoveries for oral infection. Sprent (1952) recovered 16%; Ishii (1959) recovered 10.4% Sinha and Bihar (1966) had 31.75% recover using T. canis. Oshima (1961) studied some factors which might affect larval recovery. These were: age of inoculum, adhesion of eggs to containers, inaccuracy in counting of doses, methods and apparatuses employed in the inoculation and preparation of mice to be inoculated. He found that the longer the period of storage of eggs beyond three months, the lower the infectivity. Starvation of mice before inoculation had no influence on the average total yield. In comparing two body weights of the mice on the infectivity of larvae, Oshima (1961) used 20-25 gm vs. 31-36 gm mice and there was no significant difference observed in the yields of the larvae from the two groups. The effect of removing the outer shell membranes from the eggs was also studied by Oshima. The recovery rates from deshelled eggs were higher than from normal eggs. Morphine treatment of mice 15 minutes before inoculation increased the recovery rate from 40-60 per cent. He studied all of these effects on the larval recovery at one specific time which was 44-48 hours after infection when the larvae were present in the liver. After the larvae migrate in different directions the larval recovery decreased.

The eggs that we used were not more than 3 months old. We did not

treat the eggs to deshell them or treat the mice with morphine before inoculation as we wanted to simulate a natural infection. Our mice weighed 30-35 gm. Oshima (1961) had about 45% larval recovery even with efforts to overcome factors that might affect the larval recovery. We have attributed our low recovery rates to the following factors:

1. T. canis eggs and all ascarid eggs that possess an outer proteinaceous layer are very sticky to containers. Silicone pretreatment of all equipments used in the inoculation procedure did not prevent adherence as some containers were not easily coated with the silicone suspension. Plastic tubes that were used to introduce the eggs to the stomach, and the needles and plastic syringes used to place the eggs in the stomach were not easily coated. The shape of the barrel end of the syringe attached to the needle may have prevented removal of all the egg suspension from the syringe and needle.
2. Loss through feces after inoculation: studies in mice infected with Ascaris on factors that may cause variation in number of recoverable larvae showed that part of the inoculated egg dose was lost due to passage of feces after inoculation and this involved greater proportion of a smaller dose (Jeska et al. 1969). Chari and Subramanian (1972) studied the early stages of T. canis infection in mice and found that quite a few larvae hatched but the major part of the inoculum passed through the intestine with the feces without hatching. Pretreatment of the mice with morphine before inoculation (Oshima 1961) was to increase the emptying time of the intestine. The larval recovery rate was increased from 40 to more than 60 per cent, i.e. 15-20 per cent more of the eggs hatched if they remained longer in

the intestine. Even though he did not have information about the per cent of eggs passing through the intestine without hatching, he concluded that more than half of the eggs appeared to be discharged without hatching, since he had 40-45 per cent larval recoveries using normal eggs. He also concluded that there was a difference between the hatching rate and the rate of infectivity of inoculated eggs. We confirmed the latter as 43 per cent of that part eliminated with the feces after the inoculation were hatched larvae and the other 47 per cent was eggs while only 2.1 per cent of the oral dose of _B. procyonis_ was lost due to passage with the feces. The active movement of _T. canis_ might account for the greater discharge through feces noticed for _T. canis_ but not for _B. procyonis_. Kayes and Oaks (1976) suggested that increased inoculum size could have increase the peristalsis of the intestine as a result of penetration of large numbers of larvae. This might lead to elimination of eggs and larvae in the feces.

3. The low per cent larval recovery could also be due to the larval return to the intestine after a 2nd lung migration. When larvae get in the blood they can go to any organ and might return to the lung and from there they could rupture the alveoli to ascend the trachea and be swallowed. Such larvae might again penetrate the intestinal wall or might be flushed out with the feces. The 2nd assumption i.e., larvae might be flushed out with the feces, could have been ascertained by continual collection of feces from each mouse individually to look for escaping larvae for up to 60 days. This means also that each mouse has to be put in a separate cage and that was not possible

due to limited facilities. Larval return to the intestine can explain the results obtained with the 3000 egg oral dose and the parenteral dose since we have found some larvae in the liver, lung and intestine even up to 60 days and lesions in the lung up to 17 days after infection. Larval return has been reported by Oshima (1961) and Burren (1967). The fact that we did not have an appreciable increased per cent larval recovery in the parenteral route over the oral route may support this assumption.

4. Using mice, rather than other experimental animals might be a cause for less larval recovery. Mice may act similarly to the adult dog, as they are resistent to T. canis infection. Jeska et al. (1969) found that larval recovery from the liver and lung using 1000 Ascaris eggs were highest from guinea pigs, followed by rabbits and mice and the differences were highly significant. This suggests that the number of larvae migrating between the liver and lung in mice is less than in rabbits and guinea pigs. Kayes and Oaks (1976) also suggested that an immune response may develop in the heavier infections as a consequence of the destruction of a certain number of larvae.

Using the mouse as the experimental host Sprent (1952) grouped the various ascarid species into two categories depending upon the migratory behavior of the larvae. The first category included the species that follow tracheal migration and are consequently eliminated through the intestine. This leads to temporary infection of the mouse i.e. infection of the rodent is accidental infection and this host therefore is not important in the life cycle of the parasite. A. lumbricoides and P. equorum can be placed in this category. The second category contained the species

which follow somatic migration and lead to permanent or semipermanent infection of the rodents. In this category were placed: A. columnaris, A. mustelarum, T. canis, T. leonina and T. transfuga.

On this basis B. procyonis can be placed in the second category. We have found that it encysted in mouse tissues which lead to permanent infection of the mouse. Encystment of the raccoon ascarid has been noticed by Tiner (1953a) in the thoracic viscera of the grey squirrl, particularly in the heart, lungs and skeletal muscles of the thoracic wall. The significance of encapsulation in the rodent host was considered by Sprent (1952). He interpreted their presence as a definite adaptation towards utilization of the rodent as a true intermediate host. This interpretation can be supported by the fact that raccoon, skunk and marten ascarids can become established in the final hosts when rodents containing encapsulated ascarids are eaten by these hosts (Tiner 1953a & b, 1949 & 1951). This fact and the presence of larvae of Baylisascaris in the brain tissue of rodents makes the intermediate host easily caught by the final omnivorous or carnivorous hosts, because infected rodents lose their equilibrium. This might also lead to loss of fear from larger animals (Tiner 1953a).

Tiner also found that the raccoon ascarid had a similar effect in all rodents species tested. He suggested that the raccoon ascarid is a regular cause of mortalities in small wild rodents living in raccoon habitat and that death occurred when larvae localized in the brain. Death was the result of mechanical damage to the brain.

Tiner (1953a & b) also reported that this ascarid reached up to 1600 μ in the brain. We had a maximum growth of 1290 x 65 μ in the brain on the 17th day after infection. Tiner (loc. cit.) found that the raccoon ascarid

was the most pathogenic ascarid as it grew rapidly and attained a larger size than that of the skunk or badger forms. The last two ascarids also cause death. Beaver (1956) suggested that death might be due to toxicity of the metabolic products of these larvae. It is an interesting subject and needs more investigation. Lindquist (1978) suggested that death might be due to starvation and dehydration of mice since they stopped eating and drinking when the CNS signs began. This is another aspect which deserves further research. Sprent (1973) working on the same genus but different species; B. tasmaniensis, from the tasmanian devil, found hemorrhages on the brain as a result of migration of the larvae but he did not observe CNS symptoms even though that Baylisascaris species grew also in the rodent tissues. It seems that factors other than the mechanical damage to the brain due to larger size, are causing the CNS symptoms and final death.

In conclusion we have confirmed some of other's results especially those concerning the finding that T. canis does not produce CNS signs or even grow in the abnormal host and that of their preferance for carcass and brain in later stages. That T. canis does not grow in the abnormal host such as the mouse renders the mouse and other abnormal hosts paratenic rather than intermediate. Growth of B. procyonis in the brain, intestine with cysts in the heart and diaphragm may mean that the mouse acts as an intermediate host rather than a paratenic host especially since the life cycle of this nematode in its final host has not been worked out. Our finding of B. procyonis growth and causation of deaths among mice when the oral route was used agrees with Tiner (1953a & b).

The small larval recovery might be explained in several ways. We

have surmised that larvae of both nematodes follow a chance wandering and mixed migration i.e. tracheal and somatic types of migration. The lower larval recovery and lower loss through feces of B. procyonis suggests a tracheal type of migration and eggs and larvae were lost with the feces on subsequent to infection.

In this study we have also delineated the distribution of T. canis larvae in the tissues of mice using the parenteral route and distribution of B. procyonis in the same host using oral and parenteral infection. This has not been done by other workers.

TABLE 1. Distribution of larvae among different organs at different time intervals in days after oral dosing with 500 eggs *T. canis*.

Day	R	G	K	H	I	B	C	Loss thru feces	Total	% Recovery
1	315	-	-	-	6	-	-		321	64.2
2	119	12	5	2	3	-	3		144	28.8
3	35	10	9	4	4	16	26		104	20.8
4	11	13	15	6	-	-	39		84	16.8
5	3	12	-	-	-	19	8		32	6.4
6	2	21	7	4	-	29	33		96	19.2
7	-	2	4	3	-	21	21		50	10
8	5	2	6	2	-	19	13	N. D.	47	9.4
9	-	-	-	2	-	29	-		31	6.2
11	-	-	-	-	-	19	-		19	3.8
14	-	-	-	-	-	15	-		15	5
17	-	-	-	-	-	7	-		7	1.2
20	-	-	-	-	-	4	-		4	0.8
23	-	-	-	-	-	33	-		33	0.6
26	-	-	-	-	-	54	-		54	10.8
38	-	-	-	-	-	15	-		15	3
50	-	-	-	6	-	29	-		35	7
60	-	-	-	-	-	31	-		31	6.2

R = Liver, G = Lungs, K = Kidneys, H = Heart, I = Intestine + stomach, B = Brain, C = Carcass (bones + musculature) , ND = Not done

$$\% \text{ recovery} = \frac{\text{No. of larvae retrieved}}{\text{Dose}} \times 100$$

Day = Time in days after infection.

Loss thru feces = Eggs and larvae recovered from feces during 24 hrs past infection.

Spleen was not examined.

TABLE 2. Distribution of larvae among different organs at different time intervals in days after oral dosing with 3000 eggs *T. canis*.

Day	R	G	K	H	I	B	C	Loss thru feces	Total	Recovery
1	94	5	2	-	371	-	-	143	615	20.5
2	516	5	2	7	10	2	-	134	668	22.3
3	1680	58	39	-	13	112	5	136	1985	66.2
4	95	79	18	9	6	64	67	95	433	14.4
5	45	32	36	9	11	96	51	86	336	12.2
6	25	27	18	3	4	45	47	74	247	8.2
7	18	3	17	5	4	87	93	64	290	9.7
8	17	9	19	3	6	54	73	89	270	9
9	17	-	21	8	4	49	98	76	273	9.1
11	23	4	13	3	-	24	107	181	355	11.8
14	13	17	38	16	54	31	135	96	400	13.3
17	12	13	-	3	-	81	188	55	352	11.7
20	13	-	10	5	-	69	147	68	312	10.4
23	3	-	12	2	24	85	125	56	307	10.2
26	7	11	21	-	-	95	88	65	287	9.6
38	11	7	5	2	11	125	104	73	338	11.3
50	-	21	21	9	-	93	197	93	434	14.5
60	-	14	13	13	13	113	-	72	238	7.9

Spleen was not examined.

TABLE 3. Distribution of larvae among different organs at different time intervals in days after parenteral dosing with 500 larvae *T. canis*.

Day	R	G	K	H	I	B	C	S	Total	% Recovery
1	11	192	7	-	-	2	-	-	212	42.4
2	-	93	28	3	3	10	6	-	143	28.6
3	56	93	183	24	10	20	29	6	421	84.2
4	29	72	147	36	5	48	34	14	385	77
5	22	40	64	5	4	111	8	3	257	51.4
6	51	18	24	10	-	46	24	-	173	34.6
7	14	227	74	20	5	30	28	5	403	80.6
8	10	6	5	2	2	16	52	3	96	19.2
9	-	4	5	-	-	31	31	-	71	14.2
11	7	6	3	2	2	39	25	-	84	16.8
14	8	4	20	5	2	72	64	-	175	35
17	3	4	11	7	3	76	93	-	197	39.4
20	5	5	11	11	6	59	91	21	209	41.8
23	11	4	12	25	5	33	124	-	214	42.8
26	20	6	23	3	-	48	55	4	159	31.8
38	13	11	29	-	3	68	91	-	215	43
50	21	21	32	-	-	53	83	13	223	44.6
60	31	29	53	-	-	55	88	22	275	55

TABLE 4. Distribution of larvae among different organs at different time intervals in days after oral dosing with 3000 larvae *T. canis*.

Day	R	G	K	H	I	B	C	S	Total	% Recovery
1	139	563	-	-	-	-	-	-	702	23.4
2	221	491	30	17	15	32	41	24	871	29
3	211	523	84	17	28	88	180	12	1143	38.1
4	114	399	51	6	4	119	178	4	875	29
5	321	381	113	12	15	111	104	-	1057	35.2
6	115	213	109	9	38	91	229	-	804	26.8
7	98	119	137	10	18	131	191	12	716	23.9
8	32	36	154	13	10	89	332	3	669	22.3
9	116	28	190	6	-	99	494	3	891	29.7
11	212	24	139	25	10	93	430	21	954	31.8
14	27	18	82	28	6	150	399	-	1021	34.0
17	15	34	105	32	12	114	551	29	892	29.7
20	29	31	129	29	13	192	343	14	830	27.6
23	31	40	132	31	-	112	321	9	676	22.5
26	21	16	140	4	3	121	459	6	770	25.7
38	19	29	39	7	7	147	329	13	590	19.7
50	42	14	112	19	-	123	311	21	642	21.4
60	13	12	105	-	-	112	229	-	471	15.7

TABLE 5. Distribution of larvae among different organs at different time intervals in days after oral dosing with 500 eggs *B. procyonis*.

Day	R	G	K	H	I	B	C	D*	Loss thru feces	Total	% Recovery
1	-	4	-	-	110	-	-	-	2	114	22.8
2	14	16	-	-	6	-	-	-	6	32	6.4
3	10	-	-	4^x	4	32	-	-	6	56	11.2
4	-	-	-	8^x	-	28	34	8	10	82	16.4
5	-	-	-	-	-	24	54	12	40	130	26
6	-	-	2	6^x	-	30	24	10	8	80	16
7	-	-	-	-	-	18	40	13	34	105	21
8	-	-	-	6*	-	14	22	10	10	62	12
9	-	-	-	10	-	8	26	12	25	81	16.2
10	-	-	-	12	-	8	18	8	14	70	14
11	-	-	-	12	-	18	24	9	6	79	15.8
14	-	-	-	10	-	16	18	11	10	55	11
15·	-	-	-	12	32	26	22	12	7	111	22.2
16·	-	-	-	12	18	10	32	13	6	101	20.2
17·	-	-	-	8	22	8	23	8	8	57	11.4

^{x}Free larvae

*Encysted larvae. D = Diaphragm
No larvae were found in the spleen.

·On 15th day three mice died (recovered larvae represents average of 3)
·On 16th day two mice died (recovered larvae represents average of 3)
·On 17th day two mice died (recovered larvae represents average of 3)

TABLE 6. Distribution of larvae among different organs at different time intervals in days after oral dosing with 3000 eggs B. procyonis.

Day	R	G	K	H	I	B	C	D*	Loss thru feces	Total	% Recovery
1	10	191	-	-	510	-	-	-	2	731	23.7
2	83	83	-	-	36	-	-	-	3	205	6.8
3	40	16	10	-	41	-	-	-	49	156	5.2
4	18	21	-	12	2	23	3	-	23	102	3.4
5	3	-	31	24	3	12	13	10	3	96	3.2
6	-	-	29	21	-	10	41	32	3	139	4.6
7	-	-	-	16	-	11	32	41	6	106	3.5
8	-	-	-	4	-	16	44	19	8	91	3
9	-	-	-	21*	-	16	49	13	5	104	3.5
11	-	-	-	32	62	14	6	15	2	131	4.4
14	-	-	-	27	78	13	4	28	26	170	5.6

*Encysted larvae
Spleen was not examined for larvae.
2 mice died on 9, 11 & 12 (day 12 N.D.)
5 mice died on 14th
Mice that died on the same day were digested and averaged.

TABLE 7. Distribution of larvae among different organs at different time intervals in days after parenteral dosing with 500 larvae B. procyonis.

Day	R	G	K	H	I	B	C	D[xx]	Total	% Recovery
1	-	138	-	-	-	-	-	-	138	27.6
2	-	40	-	-	-	-	-	-	40	8
3	-	4	-	6[x]	-	-	9	-	19	3.8
4	-	6	-	-	38	-	-	-	44	8.8
5	-	-	-	-	-	14	-	-	14	2.8
6	-	30	-	-	-	24	-	-	54	10.8
7	-	48	-	8[xx]	28	18	-	12	112	22.4
8	-	45	-	27	29	9	-	26	136	27.2
9	-	18	-	9	-	6	-	10	43	10.6
11	-	25	-	6	-	10	11	9	61	12.2

x = free larvae. xx = Encysted larvae
larvae were not found in spleen.

7th day One died
8th day Two died
9th day Three moribund
10th day Three died
11th day Four died
Mice that died on the same day were digested and averaged.

TABLE 8. Distribution of larvae among different organs at different time intervals in days after parenteral dosing with 3000 larvae B. procyonis.

Day	R	G	K	H*	I	B	C	D*	Total	% Recovery
1	-	591	-	-	-	-	23	-	614	20.5
2	-	79	-	-	-	-	-	-	79	2.6
3	-	9	-	-	72	-	9	-	90	3
4	-	-	-	10	15	-	11	-	36	1.2
5	-	224	-	-	-	11	-	-	235	7.8
6	-	189	-	3	39	55	-	-	286	9.5
7	-	121	-	13	-	38	-	-	172	5.7
8	-	182	-	28	35	28	-	52	325	10.8
9	-	172	-	17	36	28	-	23	276	9.2

*Encysted larvae
Larvae were not found in spleen

7th day - One died
8th day - Three died and two were moribund
9th day - Seven died
Mice that died on the same day were digested and averaged.

TABLE 9. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after oral dosing with 500 eggs *T. canis*.

Day	R		G		K		H		I		B		C	
	L*	W*	L	W	L	W	L	W	L	W	L	W	L	W
1	405	20	-	-	-	-	-	-	411	20	-	-	-	-
2	400	20	423	20	409	20	390	20	410	20	-	-	406	20
3	410	20	411	20	410	20	395	20	405	20	409	20	400	20
4	405	19	406	19	400	20	400	20	-	-	-	-	425	20
5	409	19	411	19	-	-	-	-	-	-	415	20	410	20
6	410	20	412	20	410	20	495	19	-	-	412	20	415	20
7	-	-	400	20	420	20	390	19	-	-	410	20	410	20
8	409	20	385	20	422	20	405	20	-	-	405	19	400	19
9	-	-	-	-	-	-	410	20	-	-	399	20	-	-
11	-	-	-	-	-	-	-	-	-	-	406	20	-	-
14	-	-	-	-	-	-	-	-	-	-	410	20	-	-
17	-	-	-	-	-	-	-	-	-	-	405	19	-	-
20	-	-	-	-	-	-	-	-	-	-	415	20	-	-
23	-	-	-	-	-	-	-	-	-	-	420	20	-	-
26	-	-	-	-	-	-	-	-	-	-	401	20	-	-
38	-	-	-	-	-	-	-	-	-	-	399	20	-	-
50	-	-	-	-	-	-	ND	ND	-	-	404	20	-	-
60	-	-	-	-	-	-	-	-	-	-	410	20	-	-

*Lengths and widths represent averages. Mice that died during each experiment were all digested and averaged.

TABLE 10. Lengths (L) and widths (W) in ( ) of retrieved larvae from different organs at different time intervals in days after oral dosing with 3000 eggs T. canis.

Day	R		G		K		H		I		B		C	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	404	20	411	20	409	20	-	-	405	20	-	-	-	-
2	405	20	409	20	412	20	395	20	400	20	405	20	-	-
3	400	20	403	20	403	20	-	-	403	19	411	20	401	20
4	399	20	402	20	405	20	390	20	400	19	410	20	401	21
5	406	20	396	20	400	20	402	19	397	20	404	20	398	21
6	398	20	400	20	395	19	396	20	400	20	403	20	395	20
7	401	20	402	20	394	20	400	20	395	20	400	20	392	20
8	402	20	410	21	398	20	401	20	398	20	400	20	400	20
9	405	20	-	-	400	19	404	19	405	20	401	20	400	20
11	410	20	403	19	410	20	405	19	-	-	423	-	405	20
14	400	20	402	19	411	20	400	20	405	20	405	-	400	20
17	398	20	400	20	-	-	399	20	-	-	407	19	405	20
20	400	20	-	-	401	20	393	20	-	-	400	20	403	20
23	399	20	-	-	403	20	401	20	403	20	400	20	400	20
26	401	21	402	20	403	20	-	-	-	-	397	20	405	20
38	400	20	403	20	404	20	400	20	407	20	398	20	399	20
50	-	-	400	20	402	20	400	20	-	-	400	20	398	20
60	-	-	405	20	402	20	398	20	402	20	410	20	-	-

TABLE 11. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after parenteral dosing with 5000 larvae *T. canis*.

Day	R		G		K		H		I		B		C		S	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	405	20	419	20	409	20	-	-	-	-	415	20	-	-	-	-
2	-	-	425	20	411	20	415	20	411	20	399	20	422	20	-	-
3	407	20	413	20	401	20	396	20	406	20	412	20	397	20	407	20
4	400	20	403	20	400	20	398	20	401	20	420	20	405	20	408	20
5	405	20	412	20	415	20	400	20	401	20	410	20	408	20	415	20
6	410	20	412	20	412	20	410	20	-	-	414	20	419	20	-	-
7	410	20	406	20	414	20	399	20	415	20	394	20	408	20	399	20
8	384	20	409	20	415	20	393	20	400	20	402	19	407	20	402	20
9	-	-	407	20	405	20	-	-	-	-	399	20	420	20	-	-
11	401	20	415	21	403	20	403	19	405	20	403	19	399	20	-	-
14	403	20	405	20	402	20	410	20	410	19	405	20	405	20	-	-
17	402	20	404	20	401	20	412	20	409	20	400	20	403	20	-	-
20	391	20	410	20	400	20	395	20	402	20	411	20	402	20	390	20
23	400	20	411	20	405	20	389	20	410	20	403	20	402	20	-	-
26	400	20	400	19	410	20	405	20	-	-	408	20	400	20	402	20
38	392	19	402	19	409	20	-	-	399	20	400	20	399	20	-	-
50	383	19	400	20	400	20	-	-	-	-	405	20	400	20	400	20
60	392	20	410	20	403	20	-	-	-	-	402	20	411	20	400	20

TABLE 12. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after parenteral dosing with 3000 larvae *T. canis*.

Day	R		G		K		H		I		B		C		S	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	411	20	412	20	-	-	-	-	-	-	-	-	-	-	-	-
2	413	20	415	20	415	20	400	20	405	20	397	20	410	20	400	20
3	399	20	400	20	399	20	425	20	410	20	399	20	400	20	410	20
4	410	20	395	20	399	20	403	20	405	20	400	20	415	20	405	20
5	407	20	405	20	405	20	400	20	402	20	410	20	398	20	-	-
6	410	20	399	20	402	20	393	20	411	20	405	20	400	20	-	-
7	400	20	398	20	402	20	400	20	401	20	407	19	406	19	398	21
8	400	20	410	20	400	20	403	20	403	20	410	20	407	19	410	20
9	405	20	400	20	410	20	410	20	-	-	400	20	410	20	415	20
11	401	20	406	20	401	20	402	20	404	20	403	20	393	20	400	19
14	403	20	432	20	402	20	405	20	403	20	402	20	394	20	-	-
17	401	20	399	20	394	20	403	20	402	20	403	20	395	19	402	21
20	391	20	402	20	401	20	491	20	405	20	405	20	400	20	403	20
23	393	20	404	20	401	20	389	20	-	-	403	20	400	20	405	20
26	400	20	400	20	402	20	395	20	399	20	405	20	405	20	406	20
38	402	20	402	20	402	20	410	20	401	20	400	20	402	20	400	20
50	401	20	401	20	400	20	405	20	-	-	410	20	401	20	410	20
60	400	20	395	20	401	20	-	20	-	-	405	20	400	20	-	-

TABLE 13. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after oral dosing with 500 eggs B. procyonis.

Day	R		G		K		H		I		B		C		D	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	-	-	240	13	-	-	-	-	205	12	-	-	-	-	-	-
2	202	14	239	11	-	-	-	-	201	13	-	-	-	-	-	-
3	189	16	-	-	-	-	210	12	200	16	225	13	-	-	-	-
4	-	-	-	-	-	-	283	15	-	-	305	17	236	14	295	16
5	-	-	-	-	-	-	-	-	-	-	325	18	233	14	303	17
6	-	-	-	-	290	16	312	17	-	-	355	20	255	15	350	19
7	-	-	-	-	-	-	-	-	-	-	395	24	283	17	390	22
8	-	-	-	-	-	-	429	28	-	-	456	30	295	20	432	29
9	-	-	-	-	-	-	478	30	-	-	596	35	306	19	468	31
10	-	-	-	-	-	-	535	33	-	-	496	37	310	20	530	33
11	-	-	-	-	-	-	696	37	-	-	633	40	315	21	650	37
14	-	-	-	-	-	-	730	35	-	-	725	45	314	21	620	30
15	-	-	-	-	-	-	965	53	1020	30	1005	50	316	22	939	49
16	-	-	-	-	-	-	1125	51	1155	31	1110	45	325	22	1182	43
17	-	-	-	-	-	-	1285	52	1365	29	1290	55	333	23	1205	51

TABLE 14. Lengths (L) and widths (W) in ( ) of retrieved larvae from different organs at different time intervals in days after oral dosing with 3000 eggs B. procyonis.

Day	R		G		K		H		I		B		C		D	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	201	14	210	15	-	-	-	-	228	17	-	-	-	-	-	-
2	323	20	227	18	-	-	-	-	232	16	-	-	-	-	-	-
3	320	20	273	18	233	21	-	-	331	19	-	-	-	-	-	-
4	379	20	264	21	-	-	400	26	421	20	310	19	334	15	-	-
5	398	21	-	-	334	19	390	17	420	22	460	18	449	19	375	23
6	-	-	-	-	375	22	456	21	-	-	600	26	379	19	420	21
7	-	-	-	-	-	-	560	23	-	-	690	27	335	20	564	23
8	-	-	-	-	-	-	600	25	-	-	620	27	301	18	635	28
9	-	-	-	-	-	-	700	32	-	-	723	32	310	17	657	26
11	-	-	-	-	-	-	740	33	765	25	738	29	305	20	926	28
14	-	-		-	-	-	737	44	791	31	827	41	318	20	889	45

Spleen was not examined for larvae.

TABLE 15. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after parenteral dosing with 500 larvae B. procyonis.

Day	R		G		K		H		I		B		C		D	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	-	-	220	17	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	291	18	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	280	18	-	-	410	23	-	-	-	-	231	18	-	-
4	-	-	275	17	-	-	-	-	400	25	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	410	23	-	-	-	-
6	-	-	245	20	-	-	-	-	-	-	570	24	-	-	-	-
7	-	-	205	20	-	-	525	26	425	24	623	30	-	-	440	28
8	-	-	225	19	-	-	622	27	550	23	740	29	-	-	640	31
9	-	-	235	17	-	-	895	32	-	-	810	35	-	-	675	30
11	-	-	291	19	-	-	1075	45	-	-	900	45	291	22	668	40

No larvae were found in the spleen.

TABLE 16. Lengths (L) and widths (W) in (μ) of retrieved larvae from different organs at different time intervals in days after parenteral dosing with 3000 larvae _B. procyonis_.

Day	R		G		K		H		I		B		C		D	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	-	-	219	18	-	-	-	-	-	-	-	-	280	19	-	-
2	-	-	220	17	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	275	18	-	-	-	-	310	19	-	-	305	18	-	-
4	-	-	-	-	-	-	310	20	340	20	-	-	304	17	-	-
5	-	-	300	20	-	-	-	-	-	-	321	21	-	-	-	-
6	-	-	310	16	-	-	410	22	410	22	320	21	-	-	-	-
7	-	-	280	16	-	-	530	24	-	-	352	22	-	-	-	-
8	-	-	270	17	-	-	620	27	620	27	600	31	-	-	670	27
9	-	-	240	20	-	-	750	38	750	28	692	25	-	-	814	30

No larvae were found in the spleen.

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COMPARISON BETWEEN MIGRATORY BEHAVIOR OF *TOXOCARA CANIS* AND *BAYLISASCARIS PROCYONIS* LARVAE IN ORALLY AND PARENTERALLY INFECTED MICE

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ABSTRACT

Toxocara canis, the common round worm of the dog, live in the small intestine. Second stage larvae that are contained in the eggs can cause visceral larva migrans in the abnormal host. Among those abnormal hosts are children who have pica. Visceral larva migrams can also occur in animals that are exposed to the infective eggs in the soil.

Baylisascaris procyonis is an ascarid living in the small intestine of raccoons. It has been shown that it is the cause of mortalities of small rodents living in the vicinity of the final omnivorous host. Death is said to be due to localization and growth of larvae in the brain of these rodents.

Eggs of T. canis were collected from feces, while eggs of B. procyonis were collected from female worms. Two dose levels of eggs of each parasite were used (500 and 3000). Two routes of infection were also used. Eight experiments were completed, four using T. canis and 18 mice for each experiment; and four using B. procyonis with 19 mice in each experiment. A portion of the eggs of each species were hatched and larvae were injected intravenously. Mice were killed on successive days from 1-9, then on the 11th and then every 3 days up to the 26th day. After that mice were killed on the 38th, 50th and 60th day after infection. Their organs were digested and retrieved larvae were counted in each organ separately.

Larvae of T. canis and B. procyonis were found in each organ digest. Larvae of T. canis tended to reside in carcass and brain in later days but did not increase in length or width during the period of infection. They did not cause any CNS symptoms or deaths of mice. While larvae of B. procyonis tended to localize in the brain and caused death of all of

the infected mice before 20 days post infection. The larvae noticeably grew in length and width.

Some of our results have confirmed other's in relation to the finding that T. canis did not grow, cause CNS symptoms or any deaths and that they had a predilection for carcass and brain in later days. Growth of B. procyonis and resultant death among infected mice also have been reported by Tiner (1953a & b). We have attributed our small larval recovery as well as that of others to four possible reasons and concluded that larvae of these species follow tracheal and somatic types of migration in the abnormal host.

In this study we have also delineated the distribution of T. canis larvae in the tissues of the mice using the parenteral route and the distribution of B. procyonis in the same host using oral and parenteral infection which has not been done by other workers.