ASSESSMENT OF PARATENIC HOSTS IN THE TRANSMISSION OF *Ancylostoma caninum* TO DOGS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Faculty of Veterinary and Animal Sciences
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Department of Parasitology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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1997

DECLARATION

I hereby declare that the thesis entitled "**ASSESSMENT OF PARATENIC HOSTS IN THE TRANSMISSION OF *Ancylostoma caninum* TO DOGS**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "**ASSESSMENT OF PARATENIC HOSTS IN THE TRANSMISSION OF *Ancylostoma caninum* TO DOGS**" is a record of research work done independently by **Smt. Deena Antony, U.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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DEENA ANTONY, U.

Dedicated To My

Beloved Parents and Husband

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Introduction

INTRODUCTION

The domestication of dogs by man dates back to several years and now there are around 400 known breeds. Initially dogs played the role of animal partners and also as pets. Now dogs are engaged for an array of purposes like sporting, tracking, detecting mines and drugs, policing and guiding. Perhaps there may not be any other symbiotic species which has been exploited in such a wide variety of ways.

Dogs suffer from innumerable diseases of which those caused by parasites, especially nematodes pose serious health hazards, even leading to death of the infected ones.

Ancylostoma caninum is the commonest and one of the most pathogenic nematodes of dogs. Ray *et al.* (1972) found that all the 100 dogs examined by them were harbouring *A. caninum*. This hook worm is a voracious blood sucker and according to Wang *et al.* (1983) the blood loss accounts to 43.1 (±39.6) and 12 μl per 24 hours for male and female worm respectively and simultaneously a similar volume of blood is also lost from the lacerations produced by the worm.

Juveniles of *A. caninum* infect human beings as an abnormal host causing creeping eruption or cutaneous larva migrans.

The reports on the role of paratenic hosts in the transmission of *A. caninum* in dogs, appear to be scanty though, mice (Banerjee, 1973; Bhopale and Johri, 1975) chicken (Agarwal and Johri, 1980) and cockroaches (Little, 1961) are implicated.

The present investigation was undertaken with the following objectives:

1. to study the susceptibility of albino mice, chicken and cockroaches to the infective larvae of *A. caninum*.

2. to study the migratory behaviour, fate and development of the larvae in the internal organs of the experimental subjects and

3. to ascertain the viability and infectivity of the juveniles collected from the experimental animals to dogs.

Review of Literature

REVIEW OF LITERATURE

2.1 Morphology of infective larva

Nicholas (1956) described the morphology of the infective larvae of *Ancylostoma caninum*.

Banerjee *et al*. (1970) have given the measurements of the various structures of the infective stage juveniles of *A. caninum*.

Anderson (1992) reported the length of the different stages of the larvae of *Ancylostoma caninum*.

2.2 Faecal culture

Scott (1928) obtained the infective larvae by culturing stools, mixing the faecal sample with about five times the volume of sterile sand, placing it in pans and incubating at room temperature from 10 days to 2 weeks till all the larvae became infective. Afterwards the larvae were isolated in water with a Baermann isolation apparatus.

Morgan and Hawkinds (1953) stated that though eggs hatched and produced infective larvae at temperature of 15 to 37°C, optimum temperature was 25 to 30°C. They also observed

that the eggs hatched at 42°C though, the larvae did not develop to the infective stage.

Lapshina (1955) observed that development of larvae did not occur at a temperature of 4°C and below and at 40°C and above. The effect of oxygen on the development of hookworm eggs was studied by Bandyopadhyay and Chowdhary (1963). They reported that the development and hatching of larvae were retarded in anaerobic conditions.

Balasingam (1964) observed that a temperature of 25 to 30°C was suitable for the development of eggs with the maximum hatch taking place at 25°C. Infective larvae appeared in the cultures at 25 to 35°C , the optimum temperature being 30°C. He further found that the eggs and infective larvae were readily killed by freezing temperatures.

Okoshi and Murata (1967) also found that the optimum temperature for hatching of eggs and development to the infective stage was 30°C. Soulsby (1982) stated that the infective stage of *A. caninum* reached within seven days at 23°C to 30°C and that at a lower temperature the development was more prolonged.

2.3 Experimental infection of paratenic hosts

2.3.1 Chicken

Okoshi and Murata (1968) could recover 9 per cent of *Ancylostoma caninum* larvae on the 3rd day from chicken experimentally infected with 2000 infective larvae, decreasing gradually upto 2 per cent on an average at the final stage of a 60 day period of experiment.

Agarwal and Johri (1980) reported that following an experimental infection, larval migration to the heart occurred within 4 hr, to the thoracic muscles within 8 hr, to liver, lungs and neck muscles within 12 hr and to the leg muscles with in 18 hr. They also observed that the larvae did not migrate to the kidney, spleen and central nervous system.

Agarwal and Agarwal (1981) observed that the total larval recovery from the chicken infected with 1000 larvae after 4 hrs was 84.8 per cent. The larvae migrated to liver within 12 hr and to lungs after 13 hr postinfection.

Agarwal and Agarwal (1983) noticed that chicken infected with two doses of infective larvae of A. *caninum* at 12 hr interval expelled the larvae at a faster rate than those which received the same number of larvae as a single dose.

2.3.2 Mice

Scott (1928) opined that there was individual variation in the number of juveniles recovered from organs of rats, experimentally infected with infective larvae of *A. caninum*. Matsusaki (1951) claimed that the infective larvae of *A. caninum* in rat after oral infection took the course of `stomach - small intestine-liver-lung-trachea-larynx-pharynx and finally reaching muscle' within ten days. He also found that the larvae remained alive upto a maximum of 377 days.

Lindquist (1952) experimentally infected laboratory and cotton rat with *A. caninum* and studied the histopathology of infected skin and lungs.

Soh (1958) studied the distribution and persistance of hookworm larvae including that of *A. caninum* in the tissues of mice following inoculation of infective larvae by mouth and through unbroken skin. He stated that recovery of *A. caninum* larvae after six days was higher by the oral route of infection than the cutaneous route. He concluded that the site of entry of larvae into the body influences the distribution of the larvae in the tissue.

Kida (1966) studied larva migrans in mice and frog with canine hook worm. The eggs of *A. caninum* inoculated into the abdominal cavity of mice and frogs hatched out and developed

to 2nd stage larvae but disintegrated later. At the same time 2nd and 3rd stage larvae from in vitro culture survived for longer period in frog developing upto late 3rd and early 4th stage larvae.

Banerjee *et al.* (1970) reported that after percutaneous infection in mice the infective juveniles of *A. caninum* penetrated the skin within 5 minutes of infection and after 2 hr majority of them were found in the voluntary muscles. The larvae migrated into the liver and lungs after intraperitoneal infection.

Banerjee *et al.* (1970) stated that infective juveniles of *A. caninum* given to mice orally penetrated through the intestine within 15 minutes. He suggested that stomach was not a suitable organ for the penetration of juveniles and the juveniles were able to reach the liver and muscles by oral route of infection.

Norris (1971) studied the migratory behaviour of the infective larvae of *A. braziliensis* and *A. tubaeforme* in mice by peptic digestion at intervals ranging from one day to 18 months after oral or cutaneous infection. In mice, the larvae of *A. braziliensis* and *A. tubaeforme* underwent a transient pulmonary migration between the 1st and 5th day of infection. Thereafter they were limited to the somatic musculature of the diaphragm and persisted there to a maximum of 18 months.

Olson *et al.* (1972) reported that after oral infection of mice with 20 to 25 larvae per gm body weight provided no evidence of eye invasion during the 95 day observation period although the larvae could be isolated from the central nervous system.

Migration and distribution of larvae in the tissues of mice after oral infection was observed by Bhopale and Johri (1975). They stated that mice which had been infected with 4,000 larvae survived for only six days. The number of larvae expelled or destroyed was greater in those mice infected with 2,000 larvae, whereas the percentage of larval recovery was higher from mice infected with 1,000 juveniles. None of the mice showed the presence of larvae in the gastrointestinal tract after nine days and that the migration of larvae to lungs and muscles occurred even on the first day itself.

Nature of host response to *A. caninum* larvae in the tissues was studied by Little and Beaver (1975). They found that the day after percutaneous infection, nearly all the larvae in the muscles were seen within the fibers. There was only a diffuse infiltration of inflammatory cells in response to the destruction of muscle fiber without any encapsulation or granuloma formation.

Comparative observation on the intramammary migration of *Toxocara canis* and *A. caninum* in experimentally infected mice

was studied by Min (1976). He claimed that no larva of *T. canis* and *A. caninum* were found in the mammary gland of mice infected before gestation but in those infected during pregnancy, larvae or larval fragments were found in the dialated alveoli. *Ancylostoma caninum* larvae were found free in the periglandular tissue whereas *T. canis* were encapsulated.

Bhopale and Johri (1978) studied the distribution of *A. caninum* larvae in the central nervous system of mice experimentally infected with single and repeated doses. Those that received a single dose of 4000 larvae died after six days and only a single larva could be received from the medulla. In those groups that received repeated doses of infection, larvae were present in the cerebral hemisphere, cerebellum, medulla oblongata, spinal cord and olfactory lobes.

Migratory pattern of *A. caninum* larvae in the Swiss Albino mice after subcutaneous inoculation was studied by Vardhani and Johri (1981). Infective larvae were recovered from the gastrointestinal tract, liver and lungs from those which were infected subcutaneously with 1000 or 4000 larvae and from the muscles of those infected with 500, 1000, 2000 and 4000 larvae one day after infection. They also reported that no larva was recovered at any time from the gut of mice infection with 500 larvae. By day 4 after infection, larvae

were recovered from the brain of all groups, while on day 30, the larvae were recovered only from the muscles. Maximum worm recovery from all the groups occurred on day one after infection.

2.3.3 Cockroaches

Harada (1954) suggested that Musca, Calliphora and Sarcophaga flies could carry the infective hookworm larvae and can act as passive transport host. Viable eggs of *A. caninum* were also noticed in the alimentary canal of housefly by Lapage (1956).

Little (1961) claimed that cockroaches could be successfully infected with third stage larvae of *A. caninum* by placing them on cultures containing infective larvae and by injecting the larvae into the body cavity. He stated that in the infected insects living larvae were found in the trachea, muscles, midgut body wall and malphigian tubules. Larvae in the haemocoele were found to be encapsulated by phagocytes within 1 or 2 days but those in malphigian tubules were not encapsulated and were found alive even upto 80 days postinfection. He also stated that there was no apparent development or increase in size of the larvae in any of the insect.

Miller (1970) observed that infective *A. caninum* larvae were viable and persisted in the muscle and other somatic tissues of the rat, mice, chicken and cockroaches.. Oyerindale (1976) recovered eggs and larvae of *A. caninum* from the crop, midgut and the hindgut of *Musca domestica*. The eggs recovered from the gut hatched after incubation for 48 hr. He was able to recover only larvae from the vomitus and one dead 3rd stage larva from the faeces.

2.4 Infection of pups with larvae recovered from paratenic hosts

Kida (1966) tried to infect puppies with infective *A. caninum* larvae collected from experimentally infected mice. He reported that these larvae developed to adult in the normal way in puppies eliciting an eosinophilic reaction. Miller (1970) proved mice as a potent transport hosts in the life cycle of *A. caninum*, by establishing infection in puppies with infective *A. caninum* juveniles, recovered from artificially infected mice. It was found that 15 to 22 per cent of *A. caninum* larvae persisted in the tissues of the mice and subsequently matured in the definitive host. Kelly (1977) stated that paratenic hosts like mice infected with third stage, *A. caninum* larvae may transmit infection when fed to puppies and the infective larvae may persist in the somatic tissue of mice upto 18 months.

Migration and distribution of *A. caninum* larvae in the tissues of mice orally infected with 1,000 larvae and establishment of patent infection from the mice to definitive host were studied by Mittra and Sasmal (1985a). He found that worm burden in the pups which were fed with infected mice orally was comparatively higher than in pups given cutaneous infection.

Mittra and Sasmal (1985b) also found that patent *A. caninum* infection could be established in definitive hosts by feeding infected chicks to hookworm free pups. They opined that avian tissues had only a poor adaptability to the infective larvae in comparison to mammalian (mouse) tissues.

Materials and Methods

MATERIALS AND METHODS

3.1 Collection of faecal samples

Faecal materials of dogs positive for *Ancylostoma caninum* brought for post-mortem examination at the Department of Pathology, College of Veterinary and Animal Sciences, Mannuthy were collected. Five pups experimentally infected with infective larvae of *A. caninum* were also used for obtaining positive faecal samples.

3.2 Faecal cultures

Faecal samples found positive for *A. caninum* on coprological examination were collected in sufficient quantities for coproculture. Cultures were set up using fresh filtered well water following the Modified Veglia's method (Sathianesan and Peter, 1970) and Baermann's technique.

3.2.1 Modified Veglia's method

Clean dry glass bottles measuring 10 cm in height and 3 cm in diameter were used for this purpose. Faeces was mixed well and transferred to the bottles without soiling the sides. The bottles were then closed and kept under room temperature. Aeration was done at frequent intervals by opening the bottles and removing the foul smelling gas. After 5 to 6 days,

cultures were examined macroscopically to see the movements of larvae on the sides of bottles.

The larvae were washed out from the culture bottles with filtered well water to a suitable petridish.

3.2.2 Baermann's technique

Faecal samples were thoroughly mixed with water using a mortar and pestle, then seived, and the filtrate was collected and centrifuged. The sediment was transferred to a clean Petridish to about one fourth of its capacity and covered with a suitable cover dish. They were maintained at room temperature, and aeration was done by pumping air into the medium using a pipette. The samples from the culture were examined microscopically every day to observe the development of the infective larvae. By sixth or seventh day, when the infective larvae were found to be present, they were separated from the samples by Baermann's technique. The faecal material was placed in the basket of a tea strainer wrapped in cheese cloth and immersed in lukewarm water contained in the funnel. It was allowed to stand for 12 hr by which time the larvae migrated to the water in the funnel. Since the larvae were motile, the fluid from the whole of the funnel was collected and centrifuged to recover the larvae.

3.2.3 Study of morphology of eggs/infective larvae

Faecal samples of experimentally infected pups were examined by preparing temporary acqueous mounts in well water in order to study the morphology of eggs. The larvae obtained by faecal culture were also studied for specific identity.

The specific identity of the infective larvae were determined following the details furnished by Nicholas (1956).

3.3 Experimental infection of paratenic hosts and pups

To find out the ability of paratenic hosts in the transmission of *A. caninum* to the definite hosts, trials were undertaken utilising chicken, mice and cockroaches.

Pups were also simultaneously infected with the same larval suspension to determine the infectivity of the larvae.

3.3.1 Chicken

Twenty four, day old White Leghorn chicks were obtained from University Poultry Farm, Mannuthy and were maintained in cages with poultry feed and clean drinking water.

3.3.2 Mice

Fifteen, one-month old albino mice of either sex were obtained from Small Animal Breeding Station, Mannuthy and were maintained in small cages with wet pelleted grain and clean water given ad libitum.

3.3.3 Cockroaches

Sixty cockroaches were collected locally and maintained in wide mouthed jars provided with a water bottle and fed on commercial mouse feed. The jars were covered with wire net and kept in cardboard boxes to provide shade and cool condition. Two or three cockroaches collected from each locality were dissected out and examined for any larval stage of parasites.

3.3.4 Pups

Ten pups of 6 to 8 weeks of age were purchased locally and maintained in separate cages. The faecal samples were examined directly and by faecal culture to ascertain whether they were free of infection. Infected pups were dewormed using Oxybendazole at a dose rate of 10 mg per kg body weight and the faecal samples were again examined as above to declare them uninfected.

3.4 Dose of infective larvae

The number of infective larvae in a particular sample was arrived at by examining 0.01 ml of the sample on a slide, under a coverslip and counting all the larvae present in it microscopically. The dose of infective larvae for chicken and mice were 1000 each while for cockroaches only 50 larvae were given. The pups were infected with 1500 infective larvae.

3.5 Route of infection

Infection to chicken was given by direct inoculation through mouth into the crop using a fine long pipette. The mice were also infected directly through mouth using a fine pipette. The cockroaches were divided into two groups of 30 each and each group were infected by different methods. One was by placing a small Petridish containing the larval suspension in the jar with cockroaches for a period of 10 days. The other method was to inoculate the larval suspension into the body cavity of cockroaches using a tuberculin syringe and a 27 gauge needle.

3.6 Examination of infected paratenic hosts and pups

3.6.1 Chicken

Experimentally infected chicks were sacrificed at the rate of three each at intervals of 4 hrs upto 12 hr; at intervals of 6 hr upto 24 hr; then at intervals of 12 hr upto 48 hr and at 72 hr after infection. After autopsy the crop, oesophagus, stomach, small and large intestine, liver, lungs, muscles of neck and thorax of each bird were collected separately in normal saline. The tissues of one of the chicks sacrificed at each interval were utilised for histopathological studies. The tissues of one chick each sacrificed at 18 hr and 24 hr intervals were teased carefully and the larvae recovered were counted and used for infecting pups. The larvae in the individual organs of all other chicks were isolated and counted after digestion in artificial gastric juice (pepsin 2.6 g, sodium chloride 5.0 gm and HCl-7 ml) for 12 hrs.

3.6.2 Mice

Infected albino mice were sacrificed at the rate of three each at intervals of 1, 4, 9, 15 and 30 days post infection. The stomach, small and large intestine, liver, lungs, muscles of head and neck and thoracic region of each mice were collected separately in normal saline. The larvae collected

from one mouse each sacrificed on first day and fourth day postinfection were used for experimental infection of pups. The tissues of one mouse each, sacrificed on dirst day, fourth day and ninth day and two mice each sacrificed on fifteenth day and thirtieth day postinfection were used for histopathological studies. The tissues of all other mice were digested using artificial gastric juice and the larvae counted separately.

3.6.3 Cockroaches

Cockroaches of both groups were dissected at the rate of three at an interval of 24 hr, and organs like fore gut, mid gut, hind gut, haemocoele and muscles were collected separately in normal saline. The larvae collected from cockroaches sacrificed on fourth day were utilised for experimental infection of pups. The organs of all other cockroaches sacrificed on different days were subjected to digestion with artificial gastric juice overnight and the larvae were counted.

3.6.4 Pups

Evaluation of establishment of patent infection was done by examination of the faecal sample daily from the tenth day onwards after infection. The first day on which the hookworm eggs appeared in the faeces of the pups were recorded.

3.7 Infection of pups with larvae isolated from paratenic hosts

Five pups aged 6 to 8 weeks were purchased locally for experimental infection with larvae recovered from the different paratenic hosts. They were examined for parasitic infection and infected ones were treated with Oxybendazole (10 mg/kg body weight) prior to the experimental infection and utilised for the studies. One pup was given 100 larvae procured from cockroaches two were given 200 and 300 larvae each from chicks and the remaining two were given 400 and 500 larvae from mice. Faecal samples of these dogs were examined daily from day 10 post infection and on the day eggs were discharged in the faeces, they were sacrificed and the worms collected.

3.8 Histopathology

The internal organs and tissues of chicken and mice preserved in 10 per cent formalin were cut into pieces of 2 mm size and washed in running tap water overnight. Blocks were made, sections of 3 to 5 μm were prepared and stained with hematoxylin-eosin.

Results

RESULTS

4.1 Faecal cultures

Faecal cultures were set up with faecal samples collected from dogs infected with *Ancylostoma caninum*. The cultures were maintained at a room temperature of 27 to 29°C and the infective larvae that developed within 6 to 7 days were utilised for infecting chicken, mice and cockroaches.

4.2 Morphological characters

Ancylostoma caninum egg collected from the faecal sample were oval and thin shelled with eight embryonic cells measuring 51-69 μm x 37-45 μm in size (average).

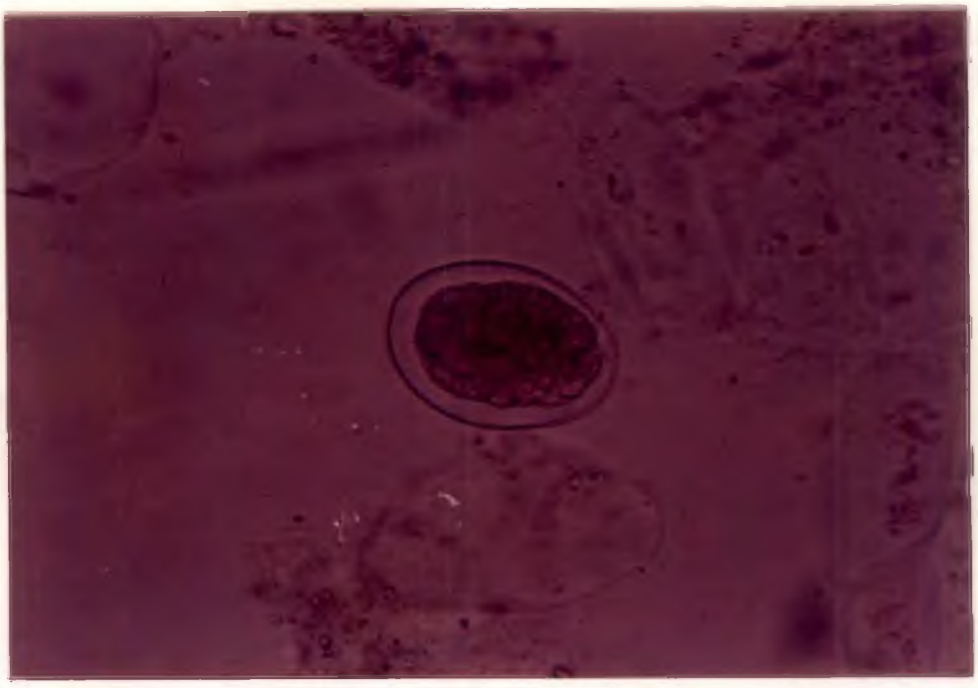
The infective larvae recovered from faecal cultures had an average length of 590 μm and a width of 21.6 μm. The length from the mouth to oesophagio-intestinal junction was 154 μm and that to the excretory pore was 122.5 μm and the distance from the anus to the tip of the body was 80 μm.

4.3 Experimental infection of chicks

The maximum total larval yield was 501 (50.1%) (Table 1) at 4 hr after infection. There after it declined sharply reaching a level of 45 (4.5%) at 72 hr after infection. Of

Fig.1 *Ancylostoma caninum* egg - Developing stage x 1000

Fig.2 *Ancylostoma caninum* larvae - Infective third stage x 400



all the organs examined at necropsy the gastrointestinal tract yielded the maximum number of larvae (215) which subsequently came down (75) considerably, though some were present in that region even at 36 hr after infection. A few larvae were present in the crop and oesophagus during the early period of infection, but none were found in those sites after 24 hr.

The earliest recovery from the liver was at 12 hr after infection. It was 14.8 per cent of the total larvae (451) recovered at that period which declined at 24 hr, to 9.5 per cent but increased to 19.4 per cent at 36 hr again declining to 10.7 per cent at 48 hr and to zero at 72 hr.

The larvae were not found in the lungs before 12 hr after infection. Highest larval recovery was effected at 36 hr (19.2%), there after declining sharply at 48 hr (13%) and none at 72 hr. There was no larval recovery from the heart, spleen and kidney of this experimentally infected chicken at any time of necropsy.

Larval migration into the muscles occurred from 12 hr onwards (9.9%). In that period larvae were recovered only from thoracic muscles. At 18 hr larvae were recorded from neck muscles also (7.8%). Highest percentage of larvae in the thoracic muscles were obtained at 72 hr (68.0%).

Fig.3 Crop of chicken 4 hr after infection - Presence of larva. H&Ex200

Fig.4 Crop of chicken 8 hr after infection - Presence of larva. H&Ex200

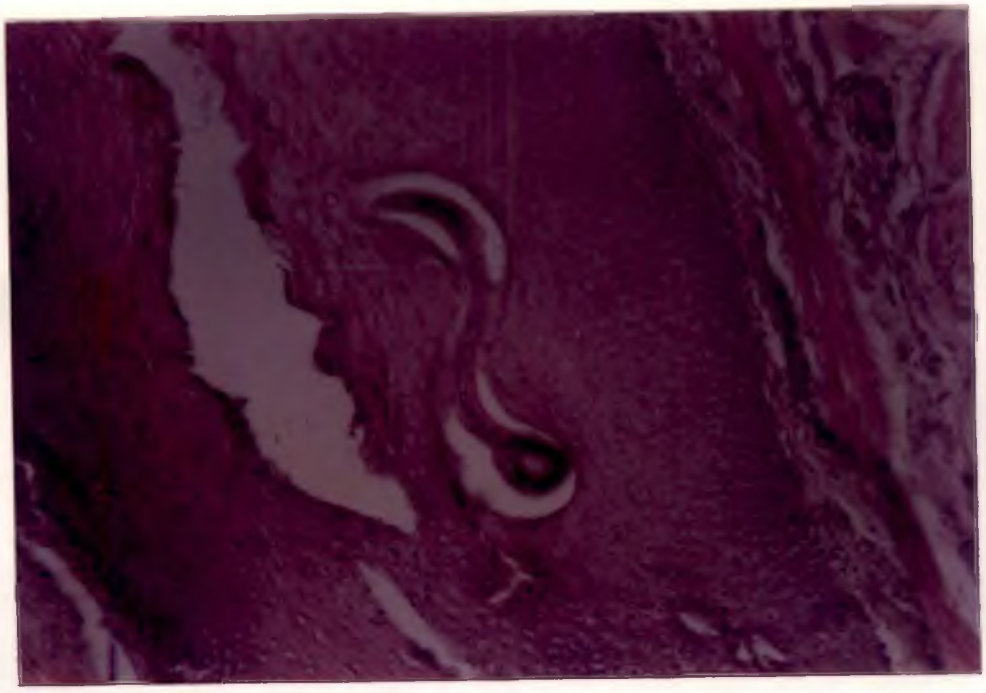
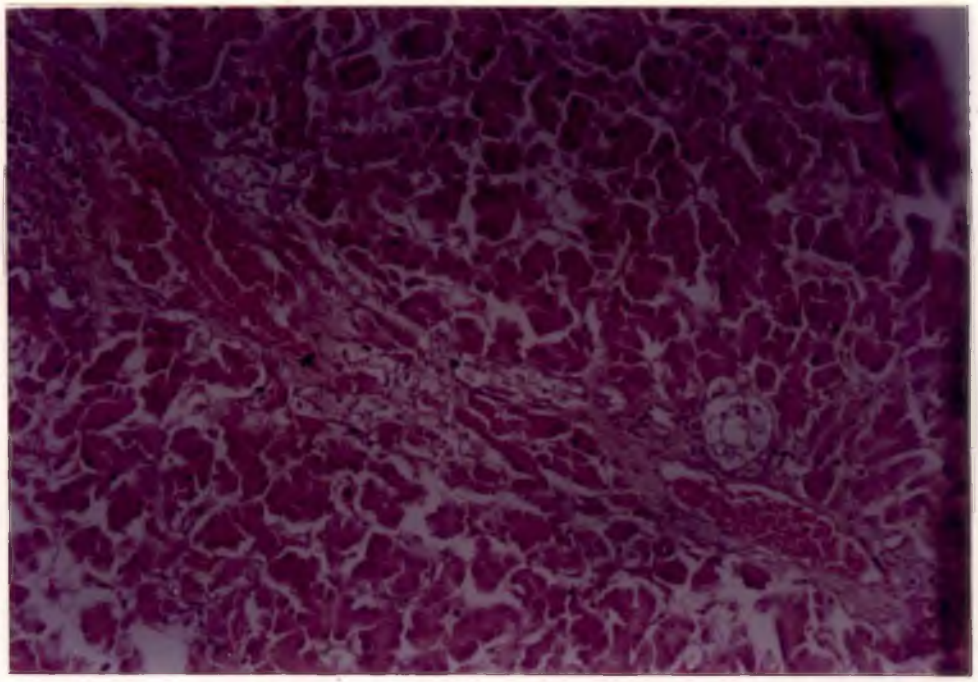
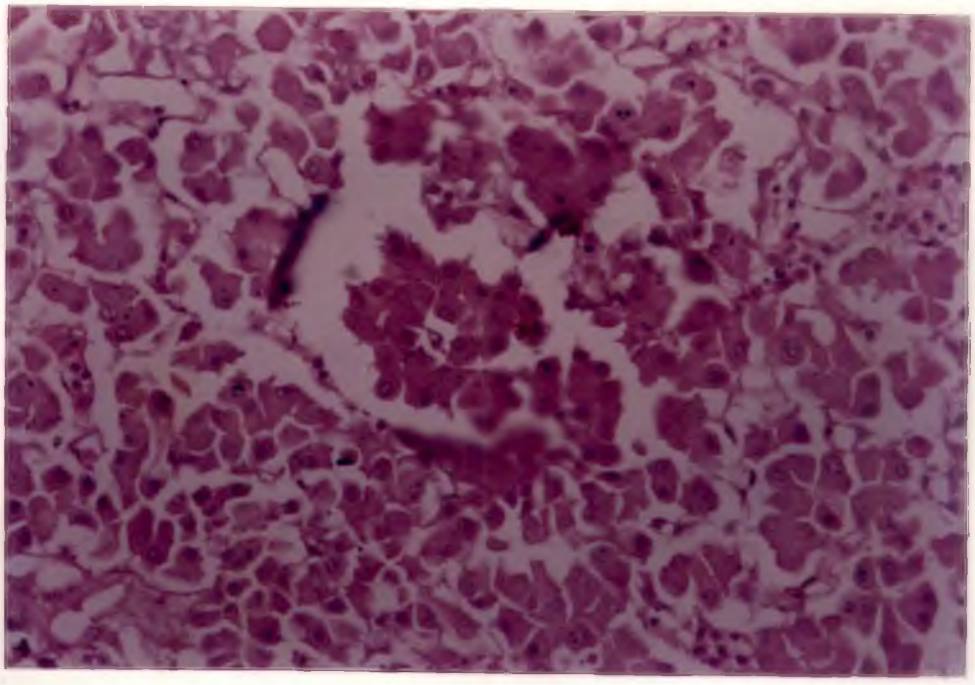


Fig.5 Liver of chicken 8 hr after infection - Congestion and infiltration of inflammatory cells H&Ex200

Fig.6 Liver of chicken 18 hr after infection - Extensive disruption and necrosis of the hepatic cells H&Ex200





4.3.1 Experimental infection of dogs

Daily examination of faecal samples collected from two dogs experimentally infected orally with the larvae recovered from chicken from tenth day onwards indicated that they were positive for *Ancylostoma caninum* eggs on nineteenth day post infection which was later confirmed by necropsy.

4.4 Histopathology of chicken

4.4.1 Crop

Sections of larvae encircled by a cystic space could be noticed. Other remarkable inflammatory changes were not detected.

4.4.2 Intestine

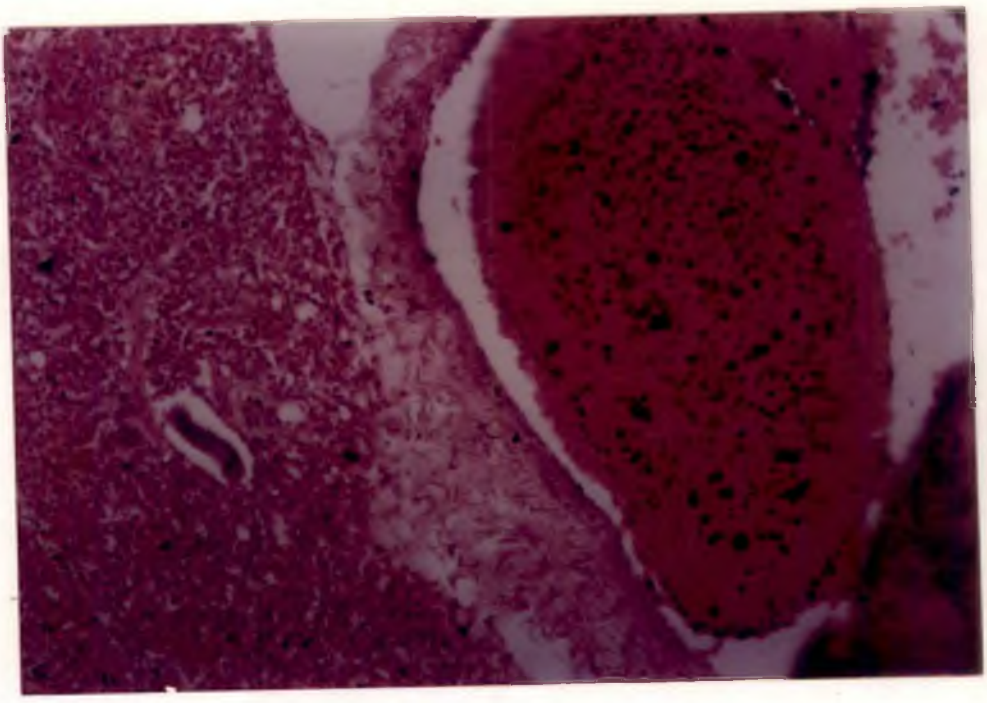
A mild catarrhal change was noticed with mild disruption of the villi.

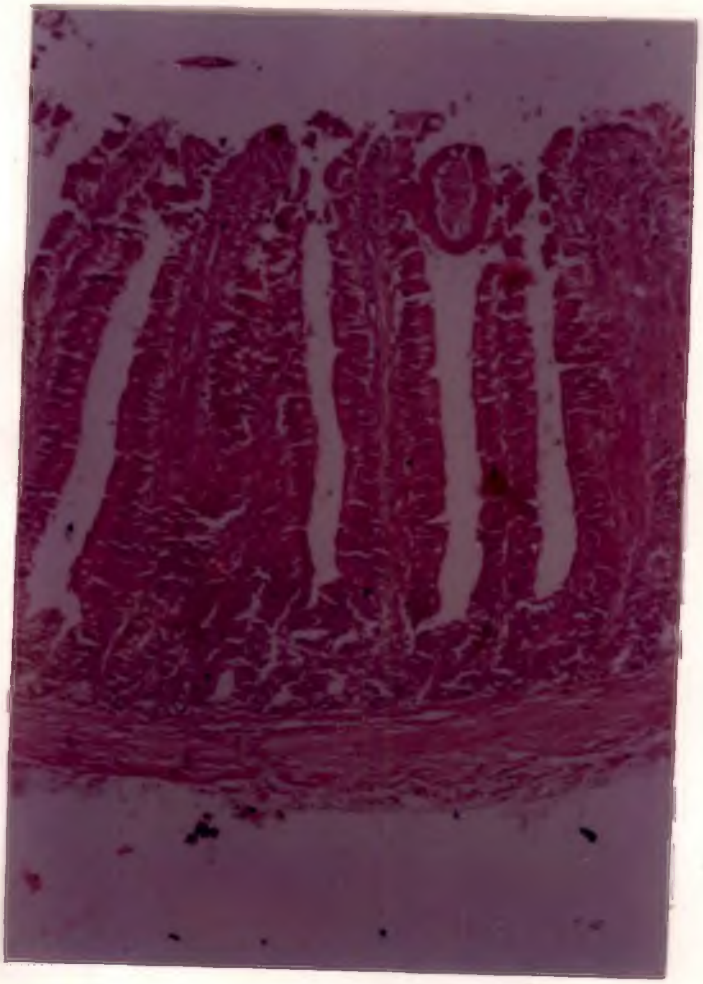
4.4.3 Liver

Area of severe congestion and vacuolar changes in the hepatic cells around the central vein were observed. Extensive necrotic areas were also present.

Fig.7 Lungs of chicken 24 hr after infection - Congestion, collapse of the alveoli and presence of larva H&Ex200

Fig.8 Intestine of mouse - One day after infection mild disruption of villi H&Ex400





4.4.4 Lungs

Severe congestion and focal inflammation were detected in the lung tissue. Sections of larvae here and there could be identified. An inflammatory zone was absent around the larval sections.

No other abnormalities were detected in any other organ.

4.5 Experimental infection of mice

Maximum total yield of larvae recorded at necropsy was 48.6 per cent (Table 2) on first day post infection.

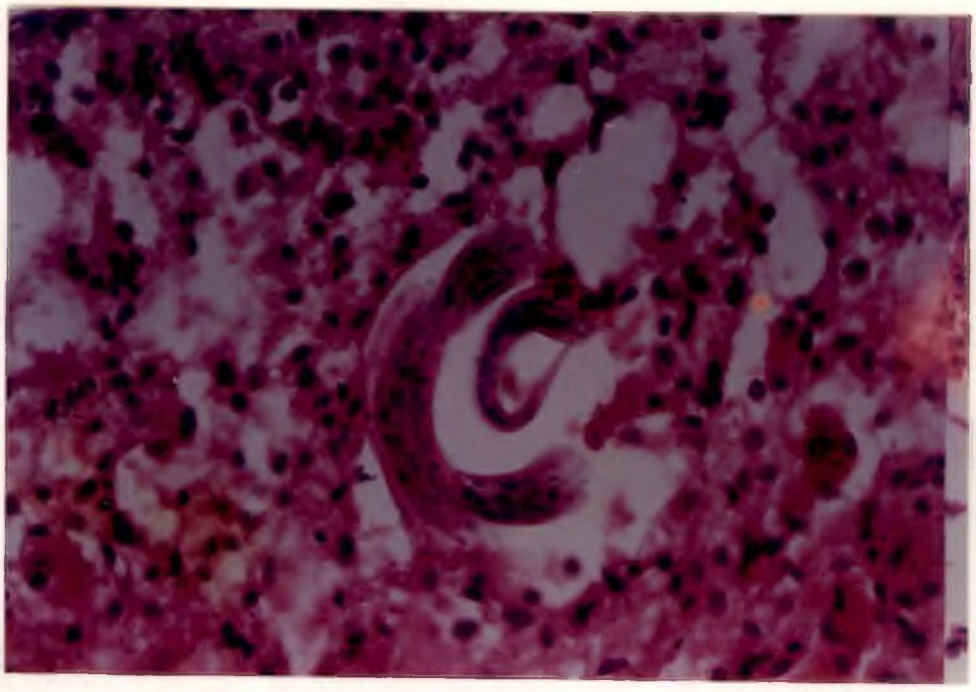
The maximum larval recovery from gastrointestinal tract of 64.38 per cent occurred on first day, which was followed by a sharp decline on fourth day and none on ninth day.

Maximum larval recovery from lungs was on 4th day (27.1%). Afterwards no larvae could be recovered from that region.

Larval migration to thorax, head and neck muscles occurred by the 4th day and the highest percentage (77.2%) was noticed on 30th day. No larvae could be recovered from spleen and kidney at any time.

Fig.9 Lungs of mouse - One day after infection - Presence of larva H&Ex400

Fig.10 Lungs of mouse - One day after infection - Presence of larva and infiltration of inflammatory cells H&Ex200



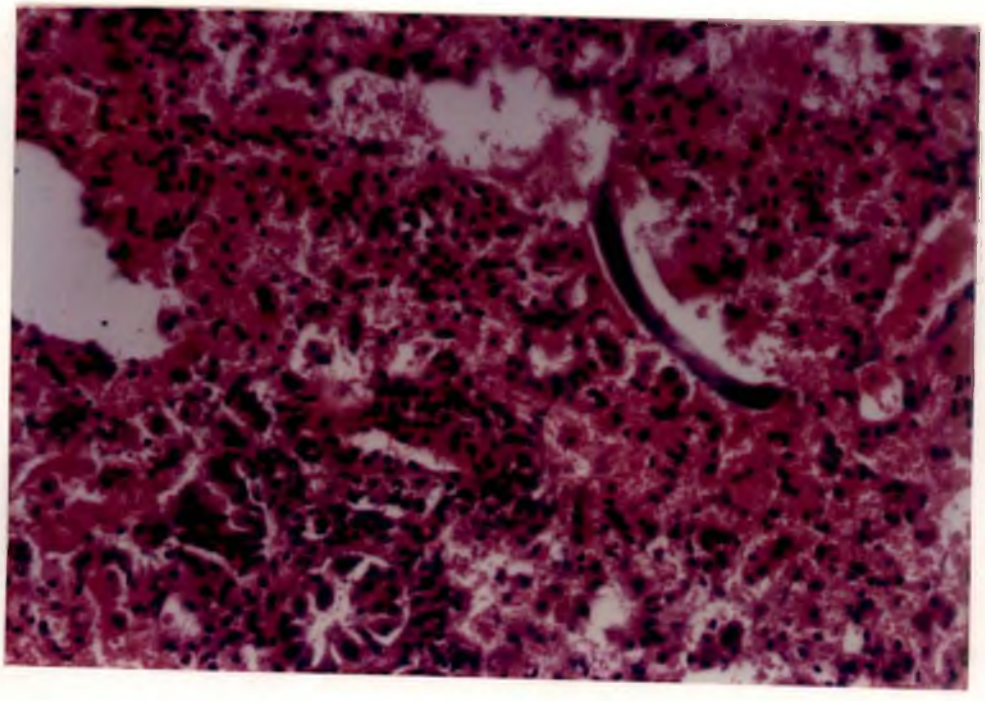
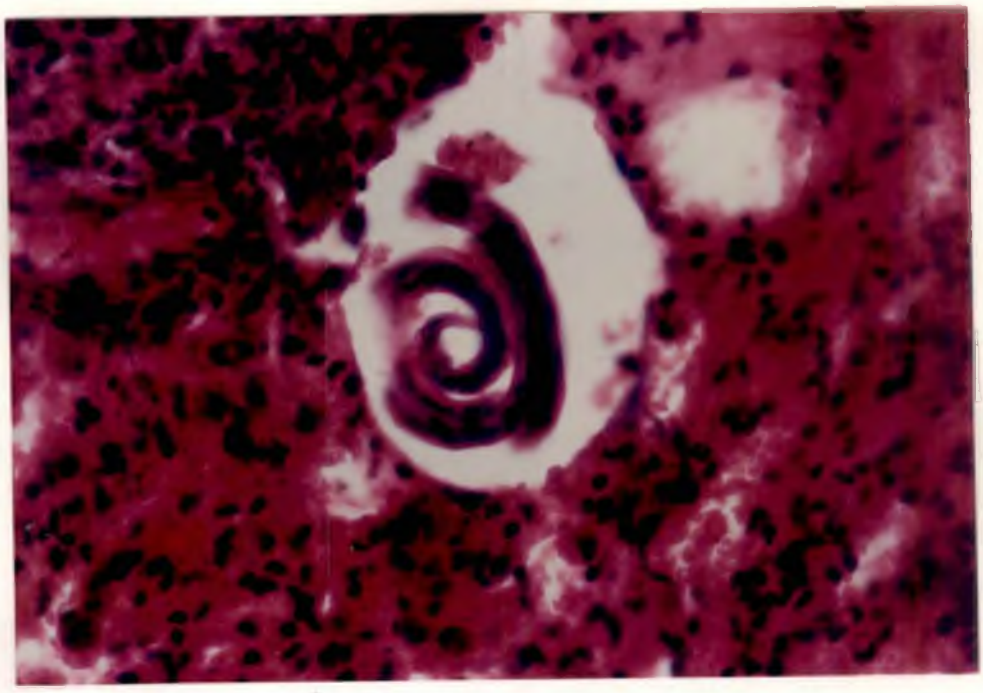
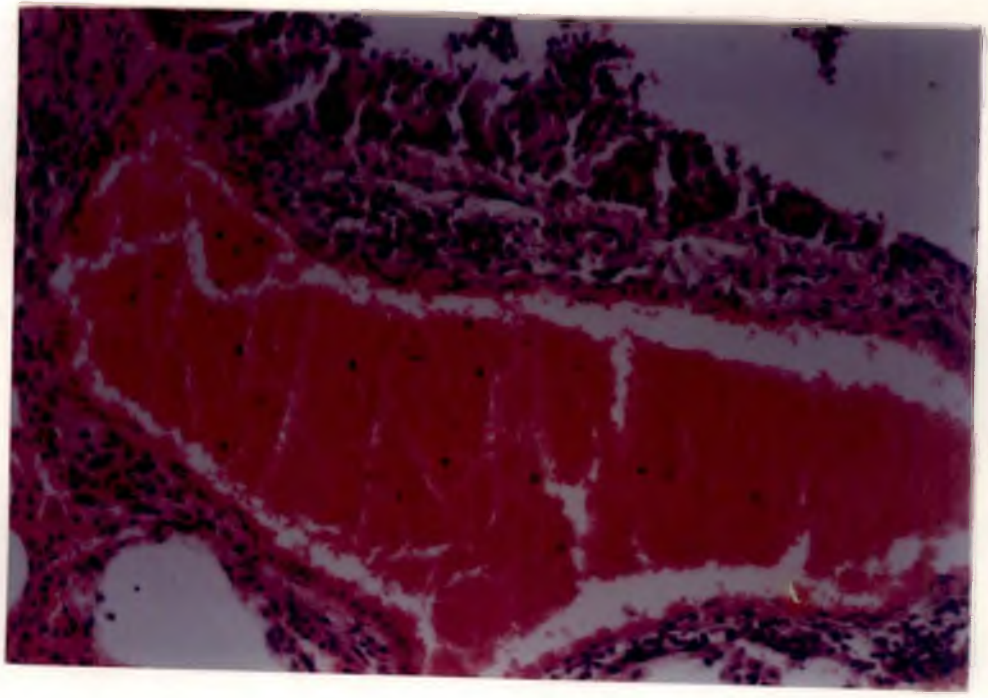


Fig.11 Lungs of mouse - Four days after infection - Haemarrhage and presence of larva H&Ex400

Fig.12 Lungs of mouse - Four days after infection - Severe congestion H&Ex400





4.5.1 Experimental infection in dogs

Examination of faeces of the infected dogs daily from the 10th day onwards showed that they discharged eggs of *Ancylostoma caninum* on 18th day post infection as was also confirmed by necropsy of these dogs.

4.6 Hostopathology of mice

4.6.1 Intestine

Mild disruption of the intestinal villi with slight infiltration of inflammatory cells were detected. Other changes were not prominent.

4.6.2 Liver

Liver showed severe congestion with focal necrosis, haemorrhage, degeneration and mild vacuolar change.

4.6.3 Lung

Sections of larvae were seen in the alveoli, rupturing the wall. Severe congestion and haemorrhage were also observed.

No other abnormalities were detected in any other organ.

4.7 Experimental infection of cockroaches

Two groups of 30 cockroaches each, one group infected by inoculating 50 larvae into the body cavity and another group exposed to faecal cultures were dissected at the rate of three at an interval of 24 hr.

On examination of 30 cockroaches which were infected per os, only 27 cockroaches showed the presence of larvae nearly in all parts of the body like the gut, haemocele and muscles (Table 3). Larvae were collected from fore gut and mid gut on the second day, from the hind gut and muscle on the fifth day and from the muscles on the sixth day after infection. Maximum yield (21) of larvae obtained from cockroaches was on seventh day post infection. Within the first few days, ingested larvae were found throughout the gut but later it was seen in the haemocele and muscles only.

In the other group, maximum number of larvae (31) (Table 4) was obtained from cockroaches infected with the infective material into the haemocele on the first day it self after infection. The number reduced gradually ending up in 7 on tenth day. Larvae were also recorded from 4th day onwards.

Fig.13 Intestine of dog infected with *Ancylostoma caninum* Inflammation, haemarrage with presence of adult worm

4.7.1 Experimental infection of dogs

Dogs infected with 100 larvae recovered from cockroaches were found to be positive after 19 days of infection which was confirmed by autopsy.

Table 1. Recovery of *Ancylostoma caninum* larvae at necropsy from different tissues of chicken infected with 1000 larvae of *Ancylostomacaninum* (Mean values derived from two birds)

Tissues/organ	Number of larvae recovered and percentage															
	At 4 hrs	Percen-tage	At 8 hrs	Percen-tage	At 12 hrs	Percen-tage	At 18 hrs	Percen-tage	At 24 hrs	Percen-tage	At 36 hrs	Percen-tage	At 48 hrs	Percen-tage	At 72 hrs	Percen-tage
Crop and oesophagas	186	37.13	159	34.95	69	15.29	58	13.03	29	6.92	-		-		-	
Stomach	86	17.16	58	12.75	52	11.53	52	11.69	22	5.25	8	2.56	-		-	
Small intestine	164	32.73	149	32.75	124	27.49	71	15.96	41	9.79	48	15.33	-		-	
Large intestine	65	12.97	89	19.56	29	6.43	57	12.81	55	13.13	19	6.07	-		-	
Liver	-		-		67	14.86	44	9.89	40	9.55	61	19.49	14	10.77	-	
Lung	-		-		63	13.97	81	18.20	73	17.42	60	19.17	17	13.08	-	
T. muscle	-		-		45	9.98	48	10.79	83	19.81	108	34.50	82	63.08	31	68.88
N. muscle	-		-		-		35	7.87	74	17.66	9	2.88	17	13.08	14	31.11
Total	501		455		451		445		419		313		130		45	

Table 2. Recovery of *Ancylostoma caninum* larvae at necropsy from differenttissues of mice infected with 1000 larvae

Tissues/organ	Number of larvae recovered and percentage									
	1 day	Percen-tage	4 day	Percen-tage	9 day	Percen-tage	15 day	Percen-tage	30 day	Percen-tage
Stomach	120	24.69	65	21.10	-		-			
Small intestine	121	24.90	5	1.62	-		-			
Large intestine	72	14.81	4	1.30	-		-			
Liver	106	21.81	10	3.25	-		-			
Lung	67	13.79	54	17.53	-		-			
Thoracic muscle	-		90	29.22	145	49.49	160	64	105	77.2
Head & neck muscle	-		50	16.23	147	50.17	90	36	31	22.8
Total	486		308		293		250		136	

Table 3. Recovery of *Ancylostoma caninum* larvae at necropsy from different tissues of cockroaches infected per os from open faecal culture (Mean values derived from three cockroaches)

Tissues/ organs	Number of larvae recovered and percentage																			
	1 day	Perce- ntage	2 day	Perce- ntage	3 day	Perce- ntage	4 day	Perce- ntage	5 day	Perce- ntage	6 day	Perce- ntage	7 day	Perce- ntage	8 day	Perce- ntage	9 day	Perce- ntage	10 day	Perce- ntage
Lumen and wall of fore gut	-	-	3	37.5	2	40	5	41.67	6	30	4	23.52	-		-		3	21.43	3	27.27
Lumen and wall of mid gut	-	-	5	62.5	3	60	7	58.33	7	35	5	29.41	6	28.57	-		5	35.71	3	27.27
Lumen and wall of hind gut	-	-	-	-					3	15	4	23.52	5	23.81	6	37.50	3	21.43	3	27.27
Haemocele	-	-	-	-					4	20	2	11.77	5	23.81	6	37.50	3	21.43	-	-
Muscle	-	-	-	-	-	-	-	-	-	-	2	11.77	5	23.81	4	25.00	-	-	2	18.19
Total			8		5		12		20		17		21		16		14		11	

Table 4. Recovery of *Ancylostoma caninum* larvae at necropsy from different tissues c infected with 50 larvae into the haemocele (Mean values derived from three

Tissues/organ	Number of larvae recovered and percentage																	
	1 day	Perce- ntage	2 day	Perce- ntage	3 day	Perce- ntage	4 day.	Perce- ntage	5 day	Perce- ntage	6 day	Perce- ntage	7 day	Perce- ntage	8 day	Perce- ntage	9 day	P n
Lumen and wall of fore gut	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Lumen and wall of mid gut	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Lumen and wall of hind gut	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Haemocele	31	100.00	23	100.00	19	100.00	10	55.56	9	56.25	10	66.67	7	50	7	58.33	6	60
Muscle	-	-	-	-	-	-	8	44.44	7	43.75	5	33.33	7	50	5	41.67	4	40
Total	31		26		19		18		16		15		14		12		10	

Discussion

DISCUSSION

5.1 Faecal culture

The development of faecal cultures of *Ancylostoma caninum* was greatly influenced by temperature. It was found in the present study that the optimum temperature required for hatching of eggs was 25-30°C. These findings concurred with those of Balasingam (1964), Okoshi and Murata (1967), Soulsby (1982) but differed from that of Morgan and Hawkins (1965) who observed hatching of eggs even at 42°C.

The present investigation showed that the time taken for the development of egg to its infective stage was 6 to 7 days at a room temperature of 25-30°C which is in agreement to that of Soulsby (1982).

5.2 Morphological characters of third stage larvae

The morphological details of infective larvae of *Ancylostoma caninum* examined during the present study were similar to those reported by Nicholas (1956), Banerjee (1970) and Anderson (1992).

5.3 Experimental infection of chicks

In the present study the maximum number of larvae recovered was 50.1 per cent at 4 hr and the minimum was 4.5 per cent at 72 hr, after infecting chicken with 1000 larvae orally. This finding more or less agrees with those of Mittra and Sasmal (1985a) but differs from that of Agarwal and Johri (1980) who recovered a maximum yield of 81 per cent of larvae at 4 hr and 7.1 per cent at 72 hr after infection of chicken with same dose and route mentioned above.

As reported by Okoshi and Murata (1968), Agarwal and Johri (1980) maximum larval yield was from the gastro intestinal tract.

In the present study, the earliest recovery of larvae from lungs and liver was at 12 hr after infection. This observation was similar to the result of Agarwal and Johri (1980) but differed from Sasada (1937) who observed lung migrating larvae at a later period of 36 to 48 hr after infection. It was seen in the present study that during the entire experimental period of 72 hr there was no mortality, of any infected chicken as stated by Agarwal and Johri (1980). They also did not encounter any mortality in any group which was infected with upto 4000 larvae. However Nicholas (1956), Banerjee *et al.* (1970) and Bhopale and Johri (1975) stressed

that mortality in their animal (mice) was due to the pulmonary haemorrhage.

Inhibition of larval migration to central nervous system, spleen, kidney and failure of recovery of larvae from these organ was in agreement with the finding of Agarwal and Johri (1980). Presence of larvae in the heart as reported by Agarwal and Johri (2.5%) could not be observed in the present investigation. This must be due to the low amount of larvae (1000) given to the experimental birds.

Concurring with the finding of Agarwal and Johri (1980) the maximum number of larvae migrated into the muscles during first 36 hr of infection. But after that, the larval burden gradually declined to an insignificant number at 72 hr after infection. The number of larvae recovered from the muscle at different time intervals was varied, irrespective of the period of examination. This indicates that the larvae may be expelled from the gastro intestinal tract soon after infection. Those that reach the muscles, after taking an extensive migratory path through the liver, lung, trachea, pharynx and muscles, appeared in small numbers since majority of them might have got destroyed during the migration. Besides, the time taken by each larvae to penetrate and migrate would be different. These features may be attributed

to the varying number of the larvae recovered from the muscles, at different time intervals.

It is apparent from present study that a significant loss of larval burden (25.5%) occurs within a very short period i.e. 72 hr post infection. This could be due to the poor adaptability of the *A. caninum* larvae to avian tissue compared to mammalian (mice) tissue as reported by Agarwal and Johri (1980) and Mittra and Sasmal (1985).

5.3.1 Experimental infection of dogs

The result of the present experiment indicate that patent infection could be established in pups with the third stage larvae recovered from chicken. This is in total agreement with the findings of Mittra and Sasmal (1985a). The prepatent period observed in the present study was 19 days.

5.4 Histopathology of infected tissues of chicken

In the absence of any earlier work in this aspect, the significance of the result obtained in the present study could not be compared.

However presence of a clear space around the juveniles in the crop was an interesting feature. Banerjee *et al.* (1970)

explained that formation of a clear space around the juveniles that penetrated into the intestinal tissue was apparently due to lysis of the surrounding host tissue caused by the activity of some enzymes or any other chemical substance liberated by the parasite.

5.5 Experimental infection of mice

The present investigation revealed that the maximum number of larvae were recovered on the first day after infecting mice with 1000 infective larvae. This is in accordance with the result of Bhopale and Johri (1975) and Mittra and Sasmal (1985b). As recorded by Bhopale and Johri (1975) and Mittra and Sasmal (1985b) there was a rapid decrease in the larval recovery from the gastro intestinal tract upto the fourth day post infection suggesting that there might be a rapid migration of larvae from the gastrointestinal tract to other tissues.

The present study indicated that the pathological changes in lungs were severe than in liver. This is in agreement with Banerjee *et al.* (1970), Bhopale and Johri (1975) and Chattervati *et al.* (1978). These workers observed numerous pulmonary haemarrhagic patches owing to the concentration of larvae in lungs.

Presence of larvae in muscles after oral infection in paratenic hosts was also reported by Soh (1958), Bhopale and Johri (1975), Mittra and Sasmal (1985b).

The larvae recovered from muscles did not show any size increase or development on the thirtieth day and thus agreed with that of Soh (1958), Mittra and Sasmal (1985) conferring the fact that mice act as paratenic host in the transmission of *A. caninum* to dogs.

Absence of larvae in spleen and kidney is in conformity with the findings of Bhopale and Johri (1975) and Mittra and Sasmal (1985b). Larvae were not recovered from the heart in the present study, though Bhopale and Johri could recover them from heart in insignificant numbers.

5.5.1 Experimental infection in dogs

It was observed that patent infection in dogs could be established with third stage infective larvae derived from mice tissue, at a dose of 300 and 400 orally. This is in accordance with the findings of Kida (1966), Miller (1970), Kelly (1977) and Mittra and Sasmal (1985b). Here the prepatent period was found to be 18 days.

5.6 Histopathology of mice tissues

5.6.1 Intestine

In the present study only a mild inflammatory change was noticed in the intestine. This is in accordance with Banerjee *et al.* (1970) and Banerjee (1973) who also explained that intestinal sections did not show any appreciable difference as compared to controls. Tissue reaction in the form of a cellular infiltration was practically absent or insignificant. This indicated that the larval penetration did not invite any tissue change except for the cellular infiltration that was observed at times in the villi, suggesting an absence of appreciable resistance in that organ.

5.6.2 Liver

In the present study liver showed severe congestion, focal necrosis, haemorrhage and vacuolar changes which were in agreement with the findings of Banerjee *et al.* (1970).

5.6.3 Lung

Present study revealed cellular infiltration, collapse of the alveoli and presence of larvae in the alveoli which concurred with the observation of Banerjee *et al.* (1970) and Lindquist (1952). Failure of cellular encapsulation around

the larvae in both the liver and lung indicated that a time interval of 24 hr, after infection might be too short a period for nodulation to start with *A. caninum*.

5.6.4 Muscle

In the present study, muscle tissue did not show any histopathological alteration concurring with that of Banerjee *et al.* (1970). The absence of any histopathological change in the infected muscle might be due to recent localisation of the juveniles in the muscle.

5.7 Experimental infection in cockroaches

In the present study third stage larvae could be isolated from gut, muscle and haemocele of cockroaches as reported by Little (1961). Maximum number of larvae recovered from the cockroaches infected with 50 larvae was 31 on first day post infection and lowest larval recovery was 7 on the tenth day post infection. A gradual reduction was noticed in the number of larvae recovered from first to the tenth day. The number of larvae recovered from cockroaches infected orally from open cultures placed in jars was 5 to 20.

In the absence of any earlier work in this aspect, the significance of the results obtained in the present study could not be discussed.

5.7.1 Experimental infection to dogs

A dose of 100 infective larvae collected from cockroaches were found capable of producing patent infection in dogs, prepatent period being 19 days. This finding concurs with the result of Little (1961) who infected mice with larvae isolated from cockroaches.

Summary

SUMMARY

Cultures of faecal samples collected from *Ancylostoma caninum* infected dogs were maintained by adopting the modified veglias (Sathianesan and Peter, 1970). The optimum temperature required for the hatching of *A. caninum* eggs was found to be 25 to 30°C and the time taken for the development of the eggs to the infective stage was six to seven days.

In order to assess the role played by possible paratenic hosts in the transmission of *A. caninum*, different doses of infective larvae were given orally to 24 chicken, 15 mice and 60 cockroaches and the migration of the infected larvae were studied by sacrificing these experimental hosts at different intervals of time. Larvae recovered from them were utilised for infecting pups for the establishment of patent infection.

Chicks infected orally with 1000 larvae were sacrificed at the rate of three each at intervals of 4, 8, 12, 18, 24, 36, 48 and 72 hr after infection. The larvae in the individual organ of all chicks were isolated and counted after digestion in artificial gastric juice. The maximum total larval yield was 501 (50.1%) at 4 hr and the minimum was 45 (4.5%) at 72 hr after infection. Among the organs examined

gastrointestinal tract yielded the maximum number of larvae (215) at 4 hr which declined to 75 at 36 hr. Larvae were recovered at the earliest from liver and lungs at 12 hr after infection at the rate of 67 and 63 respectively. Larval migration into the thoracic muscles occurred from 12 hr onwards producing an yield of 45, declining to 31 at 72 hr. Larval migration to the neck muscles occurred from 18 hr onwards producing an yield of 35, and reaching a low level of 14 at 72 hr. There was no larval recovery from the heart, spleen and kidney at any time of necropsy.

The tissues of one of the chicks sacrificed at each time interval was utilised for histopathological studies. The lesions encountered were mild catarrhal changes with mild disruption of villi in the intestine. Severe congestion and necrosis in liver and severe congestion, and focal inflammation of lung. Although sections of larvae were detected in different organs there were no inflammatory changes around them.

Albino mice administered with 1000 larvae orally were sacrificed at the rate of three each at intervals of 1, 4, 9, 15 and 30 days post infection. The maximum yield of larvae recorded on the first day post infection was 486 (48.6%) declining gradually to 136 (13.6%) on thirtieth day. Gastro intestinal tract carried the maximum number of larvae (293) on

the first day declining to 74 on the fourth day and subsequently zero. Larvae were detected in the lungs and liver within 24 hr after infection. Larval migration to thorax and neck muscles occurred by the fourth day post infection and were found there till the thirtieth day. No larvae could be recovered from spleen, kidney and heart at any time of necropsy.

Histopathological lesion included mild disruption of the villi with mild infiltration with inflammatory cells in the intestine, areas of congestion, haemarrhage and necrosis in the liver. In lungs larvae were found in ruptured alveoli.

Two groups of 30 cockroaches each, one group infected by inoculating 50 larvae into the body cavity and another exposed to faecal cultures were dissected at the rate of three per 24 hr intervals. The organs of the cockroaches sacrificed for 10 days at 24 hr interval were subjected to digestion and the larvae were counted. Maximum number of larvae (31) was obtained from cockroaches infected with the infective material into the haemocele on the first day it self, after infection The number reduced gradually ending up to 7 on the tenth day Larvae were recovered from the muscles from fourth day onwards.

In the other group which was exposed to *A. caninum* faecal culture, larvae were found only in 27 cockroaches, in the foregut and midgut on the second day, in the hindgut and muscles on the fifth day and in the muscles only on the sixth day. A maximum yield of 21 larvae was obtained from cockroaches infected per os on seventh day post infection.

A patent infection of *A. caninum* was successfully established in pups by feeding 200 to 300 larvae of *A. caninum* collected from chicks sacrificed at 18 and 24 hr, 400 to 500 larvae collected from mice sacrificed on first day and fourth day and 100 larvae collected from cockroaches sacrificed on fourth day. The prepatent period was found to be 19 days in the former and latter and 18 days in the remaining groups.

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ASSESSMENT OF PARATENIC HOSTS IN THE TRANSMISSION OF *Ancylostoma caninum* TO DOGS

By

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ABSTRACT OF A THESIS

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ABSTRACT

An investigation into the "Assessment of paratenic hosts in the transmission of *Ancylostoma caninum* to dogs" was carried out during the period from October 1996 to September 1997. The experimental animals comprised of 24 day old chicks, 15 one month old albino mice, 60 cockroaches collected locally and 10 pups of 6 to 8 weeks old.

Larvae obtained from faecal cultures set in room temperature were used for infecting paratemic hosts. The migration and distribution of *A. caninum* larvae in the tissues of chicks infected orally with 1000 larvae were studied. Larval yield at necropsy from different organs after digestion with artificial gastric juice revealed a 50.1 per cent recovery at 4 hr after inoculation followed by a sharp decline to 4.5 per cent at 72 hr. Larvae were detected in the lungs and liver at 12 hr at the rate of 67 and 63 respectively. The gastro intestinal tract yielded the maximum number of larvae (215) at 4 hr after infection. Migration in the muscles of neck and thorax was detected at 12 hr after infection. But no larvae were recovered from heart, spleen and kidney.

Migration and distribution of *A. caninum* larvae in the tissues of mice orally infected with 1000 larvae and the establishment of patent infection from mice to definite host

were studied. Highest larval recovery was at 4 hr post infection (48.6%) and lowest was (13.6%) at 30 days post infection. Migration of larvae to the liver and lungs occurred within 24 hr. No larvae were recovered from spleen, kidney and heart. Migration in the muscles of thorax and head and neck occurred within 4 days.

In the infected cockroaches, living larvae were found in the muscles, wall and lumen of the gut region and haemocele. Histopathological lesions included mild disruption of the villi with mild infiltration of the inflammatory cells in the intestine, areas of congestion, haemorrhage and necrosis were noticed in the liver and lungs with sections of larvae found rupturing the wall of the alveoli.

Patent infection was established in hookworm free pups, infected with larvae, recovered from infected chicks, mice and cockroaches and the pre-patent period was found to be 18 to 19 days. The present observations established that chicken, mice and insects like cockroaches might act as paratenic hosts for *A. caninum*, thus serving as natural sources of infection to pups.