"MIGRATORY BEHAVIOUR OF ASCARID LARVAE WITH SUBSEQUENT PATHOLOGICAL CHANGES IN RABBIT"

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR), BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In partial fulfilment of the requirements

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

(Parasitology)

By

Md. Zeyaul Hoda

Registration No. - M/V.Para/13/2005-2006.

DEPARTMENT OF VETERINARY PARASITOLOGY
BIHAR VETERINARY COLLEGE
PATNA - 800 014

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2007

In Reverent Dedicated to my Beloved Late Grand Mother "Bano"

DEPARTMENT OF VETERINARY PARASITOLOGY
BIHAR VETERINARY COLLEGE, PATNA- 14
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(SAMASTIPUR), BIHAR.

CERTIFICATE-I

This is to certify that the thesis entitled ***"Migratory Behaviour of Ascarid Larvae with Subsequent Pathological Changes in Rabbit"*** submitted in partial fulfilment of the requirements for the award of Master of Veterinary Science **(Veterinary Parasitology)** of the faculty of post-graduate studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar is the record of bonafide research work carried out by **Dr. Md. Zeyaul Hoda, Registration No.- M/V. Para/13/ 2005-2006,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

(Dr. S.R.P. Sinha)

Major Advisor

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CERTIFICATE –II

We the undersigned members of the Advisory Committee of **Dr. Md. Zeyaul Hoda, Registration No.- M/V. Para/13/ 2005-2006,** a candidate for the Degree of Master of **Veterinary Science** with Major in **Veterinary Parasitology,** have gone through the manuscript of the thesis and agree that the thesis entitled ***"Migratory Behaviour of Ascarid Larvae with Subsequent Pathological Changes in Rabbit"*** may be submitted by **Dr. Md. Zeyaul Hoda** in partial fulfilment of the requirements for the degree.

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CERTIFICATE-III

This is to certify that the thesis entitled ***"Migratory Behaviour of Ascarid Larvae with Subsequent Pathological Changes in Rabbit"*** submitted by **Dr. Md. Zeyaul Hoda, Registration No.- M/V. Para/13/ 2005-2006,** in partial fulfilment of the requirements for the Degree of Master of Veterinary Science **(Veterinary Parasitology)** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar was examined and approved on 3..../..10../2007..

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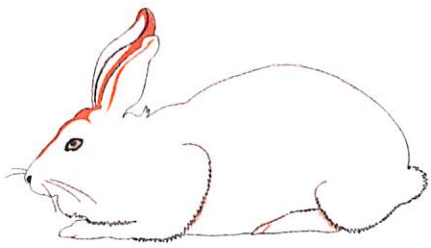

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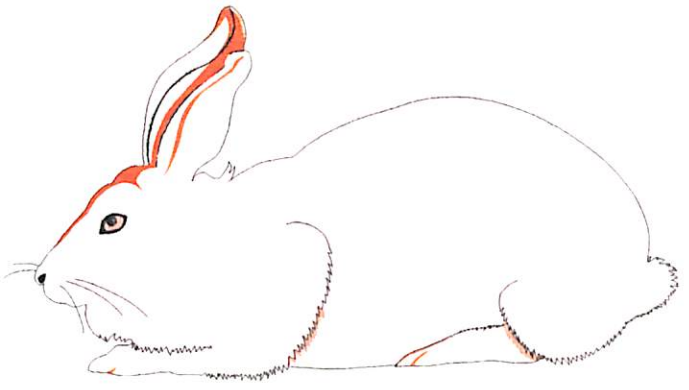

CHAPTER - I

INTRODUCTION

INTRODUCTION

Majority of neonatal pups and calves harbour the clinical grade of ascarid infection with definite symptoms of disease. The epidemiological spectrum of *Toxocara vitulorum* and *Toxocara canis* are also very wide because of their unique life cycle and their main source of infection in new born through transplacental migration of larvae which remain dormant in various organs/tissues of pregnant animals and migrate to fetus during late phase of pregnancy. The egg and the larval development of these species are determined by many extrinsic climatic factors such as temperature and humidity, therefore influence the frequency of contamination in general population of cattle, buffaloes and dogs.

Among the various helminthic infections affecting the dog, *T. canis* (Werner, 1782) is the most common and very important nematode causing ascariosis in young pups. Dogs are commonest companion to men therefore major significance of this parasite (*Toxocara canis*) is of public health point of view. It has long been recognized as an important source of helminthosis in human (Fulleborn, 1921) as *Toxocara canis* is the sole agent for causing visceral larval migran (VLM) in man especially in children (Beaver *et al.,* 1952 and Petter, 1960).

The second stage larva of *Toxocara canis*, the cosmopolitan canine round worm, was first identified as a human pathogen and etiological agent of visceral larva

migrans syndrome (Beaver, *et al.*, 1952). Further observation made by W.H.O (1981, 1987) indicated that toxocariosis of man presents a growing health problem, which requires attention and the disease has been acquired by the ingestion of soil contaminated with embryonated ova of *Toxocara canis*. Its entity is characterized by chronic eosinophilic lesion, associated with the migrating larvae in the inner organ viz. liver, lungs, brain and some time in the eye and elsewhere. Diagnosis of the syndrome (VLM) is mainly based on the patient's history, and with symptoms of the hepatosplenomegaly, transient pulmonary problems, fever, hypergammaglobulinaemia and leucocytosis associated with marked eosinophilia (Beaver *et al.*, 1952; Huntely and Beaver, 1965).

Toxocara vitulorum is responsible for calf hood mortality and constitute a vital and intrinsic problem in sub tropical countries and other parts of the world. Its main clinical sign is associated with diarrhoea and some time the ascariosis in calves may cause intestinal obstruction resulting death.

The life cycle of *Toxocara canis* and *Toxocara vitulorum* possess the typical ascarid cycle, as eggs are thick shelled and pass out in large number with faeces. These eggs are easily communicable even in slight contamination and quite resistant in various environmental conditions. The embryo once moults in the egg and turns into L_2 stage and then become infective. After ingestion in the alimentary tract emerging larvae bore

into gut wall and pass through blood stream. It migrates with blood stream to liver and for very short period through heart; then via lungs to capillaries of lung and here it grows into mature worm. During migration it ruptures alveoli and then reaches to bronchi, trachea then swallowed and again the adult worm reaches to intestine and become sexually mature. Even though some larvae remain in the blood stream and migrate into various other organs like kidney, eye, muscles etc.

It has been reported that damages done by adult worm are not much effective but migration of ascarid larvae is responsible for damage in hepatic parenchyma as degeneration of cells, swelling, necrosis especially in interlobular veins. Eosinophilia is always associated with this migration. In the lung the actual damage done by the larvae may be considerable as it consist with intra alveolar haemorrhage. Further the shedding of alveolar epithelial cells and blood cells may often cause broncho or lobar pneumonia. Migratory larvae not only cause mechanical damage to lungs but also affect the other organs as they may pass through the pulmonary capillaries to the left heart and from there-by distributed widely. They may come to rest in lymph node, thyroid, spleen or even central nervous system. Some time the larvae are killed by hostreaction and encapsulated, caeseated or calcified within the affected tissue. Apparently waste products of these large worms can be absorbed directly through the wall of the intestine and produce sensitization and toxaemia. Another

symptoms like epileptiform seizures; urticaria, bronchial asthma, photophobia, retinitis, meningitis and even haematuria are also common. Large masses of worms may become so entangled as to block the intestine, causing gangrene and death. Occasionally adult ascarid wander and enter such places as the bile duct (causing jaundice), the appendix (causing acute appendicitis), or the pancreas (causing pancreatitic haemorrhage).

As regard to damage and morbidity caused by these parasites, extensive researches on prevention and control of ascariosis are going on, but still the rate of prevalence is highly alarming and the veterinarians may not effectively bear the entire responsibility to command the key position for preventing this public health problem. It is considered that, the working knowledge on the factors responsible for infectivity through eggs, migration pattern and host reaction in various lab animals may reflect the long term effect along-with apparent changes due to parasitic infection in host body. This information would be the groundwork on which intelligent measures of prevention and eradication may be implicated. The trial will be based on experimental animal models or laboratory animal will be helpful in obtaining the answer.

Many different classes of vertebrates may serve as a paratenic (non definitive) host of *Toxocara canis* and *Toxocara vitulorum* and have been found to most closely resemble as the lab model for ascariosis (Beaver 1969).

The interference of ascaris affects vast number of people and bovine industry for failure to grow properly. Loss of weight, death are hidden or visible aspects of the diseases therefore the present project has been undertaken to study the migratory behaviour of *Toxocara canis* and *Toxocara vitulorum* larvae in rabbits, experimentally to evaluate the pathological changes in various affected organs during migration. Even on the basis of zoonotic point of view this study may provide knowledge regarding zoonotic transmission because now a days lab animals like rabbit becoming a new choice of pet and becoming a role model for experiment in various field of bioscience.

The observation of this project may lead to the myriads of scientific finding yet to be made in this direction, to hold a means of achieving success in the control and elimination of ascarid infection in calves, dogs and check the public health hazard as VLM in man by these parasites.

Considering all the above factors the present investigation has been undertaken with the following aims and objectives :-

(1) Female worms of *Toxocara canis* and *Toxocara vitulorum* were isolated from infected hosts and collection of embryonated eggs was carried out to infect the experimental animals.

(2) Migratory behavior of *Toxocara canis* and *Toxocara vitulorum* were studied in rabbits on various post inoculation days.

(3) Haematological and histopathological variations caused by larvae in host organs were studied to estimate the tissue level damage and changes caused by migratory larvae.

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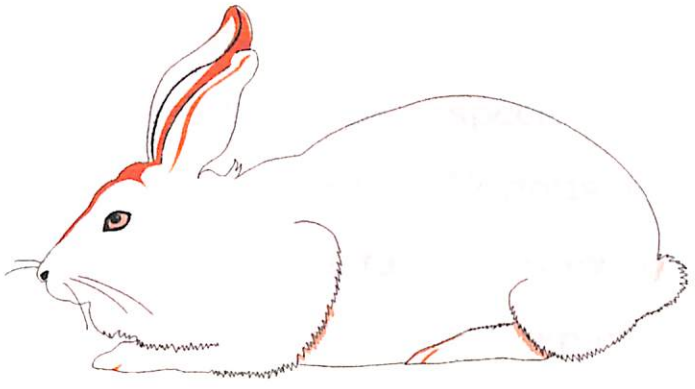

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The morphological description of the causative agent toxocariosis was first reported by Goeze (1782) and Werner (1782) in bovine calves and pups respectively. Toxocariosis is primarily soil transmitted disease. Various authors in India and abroad have presented detailed review on migration of these ascarid larvae in various paratenic hosts. However studies related to migration of *Toxocara canis* and *Toxocara vitulorum* in rabbits are scanty.

The significance of larval transmission is also in public health aspects acquires more importance along with large-scale pups and calf hood mortality. Therefore this preliminary investigation "Migratory behaviour of ascarid larvae with subsequent pathological changes in rabbit" was carried out and prevalence of toxocariasis in local population of pups and calf population was estimated further changes in haematological parameters and various tissue organs were observed during experimental infection of *Toxocara canis* and *Toxocara vitulorum* in rabbits at various days on post inoculation. Brief resume of work in this subject reported in India and abroad are as follows.

1. Prevalence of *Toxocara canis* and *Toxocara vitulorum* in canine and bovine population :

Umar *et al.* (1986) reported that faecal examination of 250 dogs in Lahore, Pakistan, during May-September, 1984,

revealed *Toxocara canis* infection in 60% of 75 stray puppies, 38% of 50 stray adults, 37.3% of 75 household puppies and 22% of 50 adult household dogs.

Anene *et al.* (1996) estimated 31.5% prevalence of *T. canis* in dogs of Nigeria.

Anwar *et al.* (1996) investigated 63.8% helminthosis in buffalo calves of Faisalabad Pakistan and recorded maximum frequency of *T. vitulorum.*

Martinez Barbadoza *et al.* (1998) recorded prevalence of *T. canis* eggs in faeces was 21.2% in pet dogs and 12.4% in stray dogs of Mexico City.

Overgauw and Boersema (1998) reported prevalence of *T. canis* was found to be 21% in adults and 48% in pups of Netherland.

Bharkhad *et al.* (1999) recorded 34.94% and 7.8% of *T. vitulorum* infestation in faecal samples of cattle and buffalo calves respectively.

Luty and Miagajska (1999) recorded that commonest parasite in dogs and cats were *Toxocara* and 31.5% and 39% respectively faecal samples were found positive for ascarid eggs.

Grover *et al.* (2000) found eggs of *T. vitulorum* in soil samples in and around Chandigarh.

Srinivasan Rao *et al.* (2000) recorded 40.09% overall incidence of *T. vitulorum* in various age groups of buffalo calves during faecal sample examination.

Rajkhowa and Hazarika (2001) recorded 37.09% incidence of *T. vitulorum* in female bovine calves of Guwahati, Assam.

Katoch (2002) evaluated 14% prevalence of *Toxocora canis* during coprological and faecal examination among pups population of Palampur.

Pratibha *et al.* (2004) observed the effect of season and studied incidence of *T. vitulorum* in cow and buffalo calves in Patna and its surrounding areas. The incidence in cow (38.33%) and in buffalo calves (41.60%) was found highest during monsoon.

Islam *et al.* (2005) recorded 42% prevalence of toxocariosis in rural bovine calves population of Bangladesh.

Sharma *et al.* (2005) recorded frequency of *Toxocara canis* infection 32.13% and 35.01% in pet and stray dogs respectively in population of Patna.

2. Studies on migratory behaviour of *Toxocara canis* and *Toxocara vitulorum* larvae (As per haematological studies) :

Brief review dealing with haematology in various lab animals experimentally infected with *Toxocara canis* and *Toxocara vitulorum* are as follows :

Panebianco (1954) observed eosinophilia up to 30% in calves after infection with *T. vitulorum.*

El-Abdin *et al.* (1975) observed a significant decrease in erythrocyte number and marked leucocytosis with

neutrophilia, eosinophilia, basophilia and lymphopenia in calves infected with *Neoascaris vitulorum.*

Hayden and Van Kruiningen (1975) revealed moderate leucocytosis and marked eosinophilia in experimentally infected dogs with *Toxocara canis.*

Butterworth (1977) observed eosinophilia in helminthic infection and demonstrated its role in immunity during helminthic infection.

Prokopic and Figallova (1982b) reported that the number of eosinophils raised on day 7 following infection in mice with *Toxocara cati* (2500 eggs/mouse) and reached maximum (28%)on day 21.The mice were re-infected with 2000 *Ascaris summ* eggs 45 days after first infection. The eosinophilic level increased again to a maximum of 26% on day 14 and remained at a higher level till day 159 after the second infection. An indirect relation was seen between the eosinophil and leucocyte levels.

Sugane and Oshima (1982) during their studies, compared peripheral blood eosinophilia between congenitaly athymic nude mice (nu/nu) and thymus- bearing heterozygous litter mates(nu/+) for 6 weeks following oral infection with *Toxocara canis* eggs. By comparing patterns of peripheral blood eosinophilic levels in nu/+ and nu/nu, 2 types of eosinophils, one T-cell dependent and other independent, were observed. The results indicated that eosinophilia is closely related to cell-mediated immune mechanism in *Toxocara canis* infected mice.

Prokopic and Figallova (1983) observed the changes in the blood picture of white mice experimentally infected with various species of ascarids. In mice given *Toxocara cati* infection (2500 eggs) raised the eosinophilia markedly from day 7; it reached 26% on day 21. *Toxascaris leonina* infection (2500 eggs) raised the eosinophilia from day 7 to a peak of about 50% on day 28. In all the group of mice eosinophil counts declined sharply after the 28th day of infection.

Jenkins and Richard (1984) observed that pups infected with *Toxocara canis* showed increasing numbers of circulating eosinophils during the phase of larval imgration through liver and lungs.

Sugane and Oshima (1984) reported that the degree of eosinophilia after infection did not show relationship with total numbers of larvae recovered from *Toxocara canis* infected mice.

Lau and Singh (1985) reported significant lower number of erythrocytes, decreased haemoglobin and packed cell volume, leucocytosis, lymphocytosis and increased eosinophilia in suckling buffalo calves with toxocariosis. However, no significant difference was observed in MCH, monocyte, basophil and neutrophil counts.

Pandey and Mishra (1985) conducted clinico-biochemical studies on anaemia associated with neoascariasis in calves, observed that there was low packed cell volume (20.24%)

haemoglobin level (6.8-7.4) gm% and TEC (4.01 – ±4.52 x $10^6/mm^3$).

Lukes (1985) studied the changes in the white blood picture during experimental larval ascariosis, toxocariasis and toxascariosis in rabbits and observed that there were marked increase in leucocytes, eosinophils and neutrophils. It was also noticed that the intensity of reaction was not related to the infection dose used and repeated infections resulted in further increase of eosinophilia.

Sugane and Oshima (1985) induced eosinophilia in *Toxocara canis* infected SJL mice. Following infection with 500 eggs of *Toxocara canis*, peripheral blood eosinophil counts in SJL mice increased and reached a peak on the 14th day of infection, then decreased. Eosinophil counts at the peak were $20000/mm^3$ blood was 43% of the total leucocytes. Mononuclear cells and neutorphils did not increase as much as eosinophils.

A study was carried out by Vossman and Stoye (1986) on clinical haematological and serological finding in puppies after prenatal infection with *Toxocara canis*. The study revealed that all massively infected puppies died within 22-49 days of age, had lower weight gain depending on the level of infection. There was more or less severe anaemia and most noticeable effect on the white blood cell count was eosinophilia, during the first week of life, which returned to normal after complication of larval migration. The causes of death were

perforation of the intestinal wall, intestinal haemorrhage and peritonitis.

Sinha *et al.* (1987) reported combined decline in percentage of neutrophils and lymphocytes with simultaneous increase in the eosinophil counts in rats fed, 2000 eggs of *Toxocara vitulorum.* The maximum decline in the number of neutrophils was recorded on day 7 post infection and of lymphocytes was on day 11-post infection. This was followed by maximum post infection eosinophilia (22%) compared to pre-infection level of only 3% at day 11 pot infection. However no significant alteration in the counts of monocytes and basophils was observed between pre and post-feeding periods of the infection eggs of *Toxocara vitulorum.*

Person *et al.* (1989) observed the haematological responses of cats experimentally infected with *Toxocara canis* larvae and recorded higher circulating eosinophil levels.

Rao and Suryanarayana (1995) studied the haematobiochemical aspect of toxocariasis in dogs. The haematological observations revealed decrease in TEC, PCV and haemoglobin but there was increase found in TLC in *Toxocara* infected dogs. Differential leucocyte count indicated with marked eosinophilia and neurtophilia in affected dogs.

Thakur *et al.* (1998) suggested that under lab condition experimental inoculation of ascarid ova show gradual development of leucocytosis with a serious eosinophilia,

anorexia, depression, weakness, incordination, motion damage and emaciation.

Usharani Devi *et al.* (2000) observed common clinical sings of randomly selected buffalo and cow calves naturally infected with *T. vitulorum.* They observed that the haemoglobin (Hb%), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC) and total erythrocyte count (TEC) of cow and buffalo calves associated with natural infection of *T. vitulorum* were significantly ($P<0.01$) lower and mean corpuscular volume (MCV) was higher in comparison to healthy ones indicating macrocytic to normocytic, hypo chromic anemia in the affected calves.

Neves *et al.* (2004) recorded that except eosinophilia there were no significant haematological changes in buffaloes infected with *Toxocara vitulorum.*

Tudor (2004) demonstrated that the experimental infection with embryonated eggs of *Toxocara canis* products decrease in RBC in the initial state till 6 day and then back to normal. Regarding WBC from the infestation time in the first hr. a decrease in total count recorded afterwards significant increase follows. High levels of comparative fluctuation were observed during migratory stages of *Toxocara canis* larvae. Eosinophilic count increases upto 6 hrs and highest level reached at 21 days post infection and then decreases gradually in Guinea pigs.

Revajova *et al.* (2006) evaluated immunoreactivity in lambs towards multiple toxocara infection and observed that, Leucocyte and neutrophill were not significantly higher 12 hrs. pi in infected animals, absolute count of eosiniphil were elevated on 14 day pi and significantly increased on day 21 in infected lambs. Greater no. of lymphocytes were observed during the course of experiment in peripheral blood. Metaboic burst of granulocytes decreased from 3-day pi. *Toxocara canis* larvae were microscopically observed in peripheral blood of lambs from 14 to 35 day pi. Multiple infections with *Toxocara canis* caused eosinophilia and increase in proliferation activity of B and T-cells and increased the production of *Toxocara canis* specific antibodies. Immune cells has enhanced killing of larvae in the peripheral blood and trapping then in granulomas.

3. Migratory Behaviour of *Toxocara canis* and *Toxocara vitulorum* larvae (As per larval recovery) :

Chauhan and Pandey (1972) observed lesions in the caecum, liver and lungs due to migration of *Neoascaris vitulorum* larvae in albino mice. But in some cases larvae migrated through the gut wall to reach liver via abdominal cavity.

Chauhan *et al.* (1974) noted identical migratory behaviour of *Neoascaris vitulorum* in albino rats and chicken.

Prokopic and Kilabanova (1980) studied the distribution of migrating larvae of *Toxocara canis* in various organs of

experimentally infected white mice. The larvae were most frequently found in liver (82%), lungs (80%) and brain (63%). The larval numbers were maximum in the liver on day 2, in the lungs on day 4, in the brain on day 14 and in the leg muscles on day 28 post-infection.

Paul *et al.* (1981) studied the migratory behavior of *Neoascaris vitulorum* in pregnant and non-pregnant mice. The observation revealed that considerable number of larvae were found in the uterus of pregnant mice between 13th to 18th days of post infection and in fetus between days 16 and 18 where as larvae were not recovered from the uterus of non pregnant mice.

Peppersack (1981) inoculated experimental infection of *Toxocara canis* in mice and reported that, the larvae were first detected in the brain after 5 days. Thereafter, the proportion of larvae in the brain gradually increased, while that in the muscles decreased.

Min (1982) found that the total recovery rate of *Toxocara canis* larvae in brain of mice increased from 0.4 to 10.6% over days 2 to 28 after infection. About half of the infective dose was eventually recovered from the brain, with the great majority of larvae being in the cerebrum and cerebellum.

Prokopic and Figallova (1982a) studied the migration *Toxocara canis* in experimentally infected mice and observed that larvae were found in liver, lungs and muscles in all groups.

Sharma and Bhatia (1983) studied migratory behavior and pathology of *Toxocara canis* larvae in chickens and albino mice. In chicken larvae were found small in number in jejunum and lieium at 6, 12 and 24 hrs and in large number in the liver from 12 and 24 hr. onwards where as in mice larvae were seen mainly in the intestine at first 24 hrs of post infection.

Sugane and Oshima (1983) found total large number of larvae was trapped in the liver after reinfection of *Toxocara canis* larvae (L_2) in mice and the number trapped increased with prolongation of the interval between 2 infections, upto 6 weeks.

Hayat and Hayat (1984) studied the migration of ascarids in lambs. They found that *Ascaris suum* larvae underwent a complete migration and 3rd and 4th stage larvae and were recovered from the lungs and intestine, while no *Toxocara canis* larvae reached the intestine and development did not proceed beyond the 2nd stage. Damage in the liver by *Toxocara canis* was more pronounced than that by *Ascaris suum.*

Abo-Shehada, *et al.* (1984/1985) studied the migration of larval *Toxocara canis* through the intestine of mice and observed that 2nd stage larvae (L_2) hatched in the stomach and within 2 hour reached all parts of small intestine, the posteriors half being the preferred site for larval penetration. Following penetration at the base of crypts of Leiberkuhn, they followed tortuous routes in the lamina propria, and entered

the tunica muscularis obliquely. Larvae were seen entering within lymphatic vessels as well as the peritoneal cavity and invasion of the vascular system followed through. Actual penetration of intestinal blood vessels was not seen.

Abo-Shehada and Herbert (1984/1985) studied the post intestinal migration of larval *Toxocara canis* in mice. Following oral infection of mice with *Toxocara canis* embryonated eggs the L_2 passed the visceral phase of migration during the first week of infection and reached the liver and lungs and peak in number in these organs in 2 and 3 days after infection, respectively, Larvae were then dispersed throughout the body and entered the myotropic neurotropic phase by the 7^{th} day of infection. The number of recoverable larvae declined gradually with periods of stable population.

Lohmann (1985) reported that the larvae of *Toxocara canis* were detected, mainly in the musles and brain of mice, at 20-160 days post-infection. A linear increase in the absolute and relative numbers of larvae in the brain was observed which correlated with the duration of the infections. Concurrently, the number of muscle larvae decreased, the decrease being more rapid in heavy larval load than in light infections. Preferential sites of larvae within the brain were not detected.

Pramanic *et al.* (1994) studied on the migratory behaviour of *Toxocara vitulorum* larvae in rabbit and observed that the larvae continuously found in the liver from 3 to 42 days p.i.

(highest on 3rd day p.i.), in the lungs from 3 to 63 days p.i. (highest recovery on day 7 p.i.) and in the muscles from 14 to 63 days p.i. (highest recovery on the day 28p.i.). No larvae were recovered from intestine, spleen and brain. They further suggested that migratory behavior of *Toxocara vitulorum* larvae and their persistence in various tissues might be related with possibility of visceral larva migran in rabbits.

Gargili *et al.* (1999) administered 5000 embryonated *Toxocara canis* egg to chickens orally and necroscopied between 2nd and 12th days after inoculation. The larvae were found in livers of all the (100%) animals. The recovery in brain and lungs was 40 to 80% respectively.

Moyo (2002) observed that when mice were infected with 500 embryonated eggs of either *Toxocara vitulorum* or *Toxocara canis*, the migratory larvae were recovered from liver on 1st to 10th day post infection however in lungs *Toxocara canis* was noted on day 1st where as *Toxocara vitulorum* recovered on day 3 p.i.

Paula *et al.*, (2005), challenged a group of mice with infective eggs of *Toxocara vitulorum* and larval count in faeces was determined along with necropsy. At three different period of challenge (7 hrs, 4 day and 30 days), highest number of larvae was eliminated in small and large intestine, liver, lungs, heart, brain, muscles of diaphragm, tongue, and quadriceps at 7 hrs. post challenge. Maximum larvae were found in large intestine. On day 4 after the challenge larvae were more often

found in the liver and lungs on day 30, number of larvae recovered only in brain and muscle.

Saeed *et al.* (2005) inoculated various doses of *Toxocara canis* eggs in arctic fox and necropsies at 150 days post infection. The highest number of larvae found in kidney. Larvae migration from the lungs to other tissues appeared to be dose dependent. The faecal egg excretion, larval burden and intestinal worm burdens decreased from the first to the second challenge infection.

4. Studies on migratory behavior of *Toxocara vitulorum* and *Toxocara canis* larvae (As per histopathological studies) :

Chauhan and Bhatia (1973) studied the migratory behaviour of *Neoascaris vitulorum* larvae in poultry. They found that the 2nd stage larvae appear in the gut mainly caecum, liver and lungs. Gross and histological examinations revealed marked congestion, inflammatory reaction at the site of larval invasion particularly in caecum.

Sinha *et al.* (1987) reported that the pathological lesion in liver and lungs were observed up to 12 days p.i. of *Neoascaris vitulourm* but by the days 13 and 25 respectively these organs appeared normal. The intestinal wall had highest concentration of larvae after 24 hrs but no larvae had detected on 3rd day during migration in an albino mice. Histopathological examination revealed emphysema of the lungs followed by acute pneumonia.

Abo-shehada and Herbert (1984-1985) demonstrated that the larvae of *Toxocara canis* in mice were seen histologically in hepatic and central veins and many larvae migrate rapidly within liver parenchyma. On day 3 reached to lungs tissues and broke them causing verminous pneumonia with peticheal haemorrhage and congestion of the alveoli. Larvae were seen in kidney cortices with evidence of active migration through the tissue.

Srivastava *et al.* (1988) conducted a study on tissues changes during post intestinal migration of histotropic larvae of *Toxocara vitulorum* histologically in albino mice, which reveals extensive degeneration of intestinal epithelium, degeneration of hepatic cells and infiltration of leucocytes along with migratory track. Median hypertrophy was also seen of some of the blood vessels. In addition, cut section of the histotropic larvae of *Toxocara vitulorum* were also present in the section of intestine, liver, lungs, kidney and spleen. These result confirm the migratory behaviour of *Toxocara vitulorum* in mice closely resemble that of *Toxocara canis* of dog.

Mondal *et al.* (2002) demonstrated pathological changes occurring in the tissues of rabbits, orally infected with 50,000 embryonated eggs to *Toxocara canis*. Inflammatory foci were observed in different organs. The migrating larvae produced gross lesions, like numerous grayish milk spots on the surface of the lungs and liver and slight enlargement and pale appearance of the kidneys and spleen. The histopathological

lesions observed in the liver were haemorrhages, nodule formation, leucocytic infiltration, necrosis and giant cell formation. The lungs showed congestion, oedema, emphysema and infiltration of neutrophils, eosinophils, macrophages and giant cells. The changes observed in the heart and spleens were congestion and haemorrhages. The small intestine had multiple nodules infiltrated by lymphoid cells. The histophatological changes in the brain included congestion, perivascular cuffing and infiltration of mononuclear cells.

Tudor (2003-04) reported the aspect concerning the macroscopic changes and the distribution of *Toxocara canis* larvae in rabbit and observed that kidney were congestive lesions at the surface and in liver yellow brownish area showed lymphocytic infiltration. The number of larvae found in the kidney correlated with larval invasion, regarding spleen there was slight increase in volume since the beginning days of post infection and there was no changes found in the brain.

Tudor *et al.* (2004) reported anatomo-pathological changes of infestiation of *Toxocara canis* in Guinea pig and observed small haemorrhages in intestinal mucous membrane as first modification. In liver brown yellow spot appears on the surface, which also persists on section.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present experimental studies was conducted in following steps : -

(1) **The Collection of the parasite :**

(a) Faecal samples of 169 calves, aged between 20 to 50 days, situated within the municipal area of Patna were screened out for the presence of *Toxocara vitulorum* eggs. The calves found positive for toxocariosis, treated with anthelmintic, piperazine adipate at the rate of 250 mg/kg of body weight and the collection of the parasite was carried out on next day during defaecation.

The adult worms *Toxocara vitulorum* were also collected from slaughter buffalo calves located in the various abattoirs in Patna.

(b) Faecal samples of 147 (both owned and stray) pups were screened out for the presence of *Toxocara canis* ova. Out of 62 positive cases, 5 (five) pups were sacrificed for collection of the adult worm.

(2) **Identification of the parasite :**

The collected worms of the both *Toxocara vitulorum* and *Toxocara canis* were cleared in physiological saline and the adult female worms were separated on the basis of morphological studies.

(3) **Procurement of the egg :**

The uteri of the both *Toxocara vitulorum* and *Toxocara canis* adult female parasites were scooped out, grinded in mortar and pestle and filtered through nylon sieves to obtain more eggs.

Egg. Culture : -

The eggs (*Toxocara vitulorum* and *Toxocara canis* separately) obtained by this method were kept in petridishes containing thin film of physiological saline enough to keep the eggs in suspension. The eggs of *Toxocara vitulorum* were then incubated in B.O.D incubator at the temperature ranging from 37-38^{0}C where as eggs of *Toxocara canis* were incubated at 27^{0}C.

A few drops of 0.5% formalin solution were added to these petridishes to prevent the growth of any fungus in the culture. The petridishes were kept a bit open to allow aeration during incubation. The suspensions contained in petridishes were shaken daily and physiological saline was added according to necessity. Further small amount of suspension was daily observed under microscope to assess the stages of embryonation of eggs during culture.

The eggs of *Toxocara vitulorum* were developed into infective stage in 53 days where as eggs of *Toxocara canis* took 14 days to reach the infective stage.

(4) **Experimental animal used :**

Eighty (80) rabbits of New Zealand white breed having average age of 6 month and weight ranged between 900 gm to 1100 gm were purchased from local market of Patna and their faecal samples were examined to ensure the worm, ova or cyst free condition. Out of negative cases 55 rabbits were selected as per the experimental plan.

Experimental rabbit were maintained under proper hygienic condition with balance diet during whole period under study. These 55 rabbits were randomly divided into three groups. First group containing 5 rabbits were kept as control (uninfected) Group – (I), where as Group-(II) and Group – (III) which consisted of 25 rabbits in each group, were selected for the administration of *Toxocara vitulorum* and *Toxocara canis* infection respectively.

(5) **Standardization of dose :**

After thorough shaking of egg suspension, 0.1ml was taken out on a clean microscopic slide and the embryonated eggs were counted under low power of microscope. An average of 15 samples was worked out. The number of eggs counted and the standardization of dosing was maintained either by adding water or by discarding supernatant fluid, and finally strength of (500 eggs/0.1ml) of suspension was maintained.

(6) **Inoculation of infection :**

Inoculation of infection was carried out with calibrated syringe (1ml) and uniform dosing of infection of *Toxocara vitulorum* and *Toxocara canis* (i.e. 5000 embryonated eggs) was decided to administer orally in Group-II and Group-III respectively. The infection was provided to each animal of both group-II and group-III.

(7) **Study on migration pattern :**

Therefore investigation of migration pattern was studied in 55 rabbits as divided into following groups.

Group-I	Healthy control consisting of 5 rabbits (remained un-infected)
Group-II	Consisted of 25 rabbits, inoculated with *Toxocara vitulorum* infection.
Group-III	Consisted of 25 rabbits inoculated with *Toxocara canis* infection.

(I) Hematological studies :

Migratory pattern of the larvae of *Toxocara vitulorum* and *Toxocara canis*, in respect to various haematological parameters were carried out on 2nd, 7th, 14th, 21st and 30th days of post inoculation of infection.

To study the changes in the blood parameter in experimental animals infected with *Toxocara vitulorum* and *Toxocara canis* infected group as well as in control group, peripheral blood was collected before sacrifice on

all observation days (The blood was collected from ear veins with the help of syringe and taken in a anticoagulant (EDTA) containing vials.)

Following haematological parameters were determined as per standard method described by Schalm *et al* (1975).

(a) Haemoglobin in percentage (Hb %).

(b) Packed cell volume (PCV).

(c) Total leucocytes count (TLC).

(d) Differential leucocytes count (DLC).

(II) Larval count :

The liver, Lungs, kidney, spleen, muscles tissue of control and infected animals were collected after sacrifice as per experimental plan and half of these organs were processed with pepsin digestion method to study the presence of larvae, and the rest half portion of these organs were left for histopathological studies.

Digestion technique :

The tissues were cut with scissors and were grinded in a warring blender by adding 10ml of physiological saline for 30 seconds. Thereafter, the blended tissues were digested for 3-4 hours at 37^{0}C in pepsin-acid-solution consisting of 0.5% pepsin w/v and 0.7% HCL V/V in water. For musculature, 200ml solution was used but for other

organ, 100ml solution was used, larvae were collected from the digested tissue using Baermann's technique and the aliquots were sedimented by centrifugation. A portion of sediment was then transferred from a graduated centrifuge tube on large glass slides with a pasture pipette and the larvae were counted under the lower magnification of the compound microscope. The total count of the sediment for each half of the organs was multiplied by two (2), to get the total number of larvae from that organ.

(III) Histopathological studies :

As per experimental plan 5 rabbits from Group-I sacrificed at commencement of investigation and five rabbits from Group-II and Group-III were sacrificed at 2^{nd}, 7^{th}, 14^{th}, 21^{st} and 30^{th}, post inoculation days of experiment. Various infected organs were collected, processed and observed for larval count, gross pathological changes and preserved in formalin for histopathological studies.

Rest half of the organs other than digestion viz. liver, lungs, kidney, spleen and muscles tissues, of control (Group-I) and infected groups (i.e. Group-II and Group-III) were fixed in 10% neutral buffered formal saline (pH 7.0). After fixation these tissues were washed

overnight in running tap water to remove traces of formalin.

Dehydration of tissue was carried out by passing through ascending grades of alcohol. Then these tissues were cleared in two changes of xylene. After clearing, the tissues were infiltrated with paraffin wax in an oven. Separate blocks were prepared after proper embedding. The sections were cut on rotatory microtomes at 4 to 6 microns (μ). The cut sections were placed in a water bath at about 50°C to flatten the tissue and then placed on glass slides, smeared with Mayer's egg albumin and then slides, were kept in inclined position on glass rods and dried.

The sections were stained with Haematoxylin and Eosin adopting the methods of Lillie (1965) for routine and histopathological studies.

(IV) Statistical Analysis :

The data, thus collected was tabulated and statistically analysed using standard statistical techniques (Snedecor and Cochran, 1967).

Experimental schedule to study the migration and distribution of *T. canis* and *T. vitulorum* larvae in rabbit.

Sl. No.	Day of sacrifice	No. of rabbits sacrificed			Comparative observations	
		Gr-I	Gr-II	Gr-III	Haematological parameters estimated	Gross and histopathological studies
1.	2nd	5	5	5	TLC DLC PCV Hb%	Liver, Lungs, Kidney, Spleen and Muscles tissue
2.	7th		5	5		
3.	14th		5	5		
4.	21st		5	5		
5.	30th		5	5		
Total	5 observa-tion	5	25	25		

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CHAPTER - IV

RESULTS

RESULTS

The present work embodies the results of *"Migratory behaviour of ascarid larvae with subsequent pathological changes in rabbit"*, in respect to larval recovery, haematological and histopathological changes due to migration of *T. canis* and *T. vitulorum* in various affected organs of rabbits on different days of post infection.

PREVALENCE OF TOXOCARIOSIS IN CALVES AND PUPS POPULATION OF PATNA

(i) To examine the over all prevalence of toxocariosis in bovine calves, 169 faecal samples were collected and examined. It was observed that 80.47% of calves population were having clinical level of toxocariosis as heavy egg burden was detected from faecal samples.

(ii) Similarly faecal samples of 147 owned and stray pups of nondescript/descript breed were collected from Patna district and examined to evaluate the frequency of toxocariosis in local newly born stray pups population. It was recorded that 42.17% of pups were infected with toxocariosis.

Over all 316 faecal samples were examined in present investigation, and 62.65% various ascarid infections was noticed in local bovine and canine population.

Collection of Female worms:

Variation in worm recovery in respect to sex was found to be non-significant both in case of bovine and canine neonates, as average 5 male and 4.6 female worms were recovered from intestine of 5 stray pups where as average, 7.4 and 6.4 male and female worms respectively identified on examination of collected worms from 5 bovine calves on administration of anthelmintics.

Haematological observation during larvae migration of *T. vitulorum* in rabbits:

Haemoglobin : Mean ± S.E. along with CV% of Haemoglobin percent in rabbits inoculated with *T. vitulorum* infection at different days of interval have been presented in table-4 and in the figure 1. The average estimated values of Hb% in control group was recorded to be 11.32 ± 0.05 gm percent, which was found to be decrease significantly ($P<0.05$) in infected rabbits in subsequent days of interval up to 21st day then slight increase was found on 30th day of post infection.

The analysis of variance revealed significant ($P<0.05$) influence of *T. vitulorum* infestation on blood Hb% in rabbits. The average estimated values of Hb% on 2nd, 7th, 14th, 21st and 30th day were observed to decrease by 0.56, 0.62, 1.16, 1.52 and 0.5 gm% respectively than the control group (Table 4). However differences were non-significant among the estimated values on 2nd, 7th and 30th day. The lowest average estimate of Hb, was 9.80g % observed on 21st day.

Total leucocyte count (TLC) : The mean ± S.E. with CV% of TLC at different days of interval have been presented in table-4 and in the figure 1. The average estimated value of TLC in control group was 8.03 ± 0.03 ($10^3/mm^3$), which was found to be increased significantly ($P<0.05$) in infected rabbits in subsequent days of interval up to 14th days of observation, and then slight decrease was found up to 30th days of observation.

The analysis of variance revealed significant ($P<0.05$) influence of *Toxocara vitulorum* infestation on blood TLC values in rabbits. The average estimate of TLC on 2nd, 7th, 14th, 21st and 30th day was observed to be increased by 1.75, 3.57, 6.22, 5.14 and 3.03 ($10^3/mm^3$) respectively than the control group (Table 4) while differences were significant among the estimated values on 2nd, 7th, 14th, 21st and 30th days of observation. The lowest average estimate was observed on 2nd day 9.78 ($10^3/mm^3$) of observation

Packed cell volume : The mean ± S.E. with CV% of PCV in rabbits at different days of interval have been presented in table-4 and in the figure 1. The average estimate of PCV in control group was estimated to be 34.05 ± 0.16% which was found to be decreased significantly ($P<0.05$) in infected rabbits in subsequent days of interval up to 30th days.

The analysis of variance revealed significant ($P<0.05$) influence of *Toxocara vitulorum* infestation on blood PCV values in rabbits. The average estimate of PCV on 2nd, 7th, 14th, 21st and 30th day were observed to be decreased by 0.18, 0.11,

0.74, 1.53 and 0.95 respectively than the control group (Table 4), However No significant difference was observed between days 2nd and 7th and also among 14th, 21st and 30th post infection days of observation. The lowest average estimate was observed on 30th day (33.10%).

Differential Leucocytes Count (DLC) :

Original mean values of various leucocyte and average of its corresponding arc sin ± S.E. along with CV% are presented in Table-5

Lymphocyte : The mean its corresponding arc-sin value ± S.E. with CV% of lymphocyte at different days of interval have been presented in table 5 and in the figure 2. The average values of lymphocyte in control group was estimated to be 46.26 ± 0.33 percent which was found to be decreased significantly ($P<0.05$) in rabbit up to 21st days then slight increase was noted on 30th days of observation.

The analysis of variance revealed significant ($P<0.05$) influence of *Toxocara vitutorum* infestation on blood lymphocyte values in rabbits. The average estimate of lymphocytes on 2nd, 7th, 14th, 21st and 30th observation days were observed to decrease by 1.95, 5.40, 8.09, 7.62 and 3.1 respectively than the control group (Table 5). However the differences were significant among the estimated values on 2nd, 7th, 14th, 21st and 30 days of observation. The lowest average estimated was observed on 14th days of observation (38.17%).

Neutrophils : The mean its corresponding arc-sin value ± S.E. with CV% of neutrophil at different days of interval have been presented in table-5 and in the figure 2. The average values of neutrophil count in control group was estimated to be 40.74 ± 0.29 percent, which was found to be increased slightly on 2nd days of observation, then significantly ($P<0.05$) decreased in subsequent days of interval up to 21st day then slight increase was found on 30th days of observation.

Analysis of variance showed highly significant ($P<0.05$) influence of *Toxocara vitulorum* larval migration on neutrophil counts in rabbits. Average estimate of neutrophil on 2nd, 7th, 14th, 21st and 30th days were observed to be increased/ decreased by + 0.46, 3.52, 3.28, 1.39 and 0.46 respectively than the control group (Table 5). However differences were non-significant among the estimated values on 7th and 14th day. The lowest average estimated was observed on 7th (37.22%) days of observation.

Eosinophils : The mean its corresponding arc-sin value ± S.E. with CV% of eosinophil at different days of interval have been presented in table-5 and in figure 2. The average estimated value of eosinophil in control group was recorded to be 7.54±0.81 percent, which was found to be increased significantly ($P<0.05$) in subsequent days of interval up to 14th days then slight decrease was found upto 30th day of observation.

Analysis of variance showed highly significant ($P<0.05$) influence of *Toxocara vitulorum* infestation on blood eosinophil values in rabbit. The average estimate of neutrophil on 2nd, 7th, 14th, 21st and 30th day were observed to be increased by 4.76, 16.95, 19.86, 17.1 and 8.42 respectively than the control group (Table 5). However the differences were significant among the recorded value of eosinophils on 2nd, 7th, 14th, 21st and 30th day. The lowest average estimate was observed on 2nd days of observation (12.30%).

Monocytes : The mean corresponding to arc sin along with standard error with CV% of monocytes at different days of interval during migration of *T. vitulorum* larvae have been presented in table 5 and in the figure 2. Average estimated monocyte values in control group were 9.92%, which was non-significantly decreased by 0.37% on 2nd days of observation. However estimated value of monocyte further increased by 0.30% on 7th and 14th and 30th day of observation from control group but this difference was also found to be non-significant ($P<0.05$) during analysis of variance and estimated values on 21st was also similar to initial observation (control group). It was evident from the table-5 and in the figure-2 that there was no significant difference observed throughout the experimental period in rabbits infected with *T. vitulorum* infection.

Haematological observation during larvae migration of *Toxocara canis* in rabbits :

Haemoglobin : The mean ± S.E. with CV% of Haemoglobin percent on different days of interval have been presented in table-6 and in the figure-3. The average values of Hb% in control group was estimated to be 11.32 ± 0.05 (gm %), which was found to be decreased significantly (P<0.05) in infected rabbits in subsequent days of interval up to 21st days then slight increase was seen on 30th day.

The analysis of variance revealed significant (P<0.05) influence of *T. canis* infection, on blood Hb% in rabbits. The average estimate values of Hb% on 2nd, 7th, 14th, 21st and 30th day were observed to be decreased by 1.64, 2.46, 3.24, 1.84 and 0.64 respectively than the control group(Table 6). However the difference was significant among the estimated values on 2nd, 7th, 14th, 21st and 30th days of observation. The lowest average estimate of Hb was (8.08gm %) observed on 14th day of post inoculation.

Total leucocyte count (TLC) : Mean ± S.E. with CV% of total leucocyte count (TLC) at different days of interval have been presented in table-6 and in the fig.3. The average value of TLC in control group was estimated to be 8.03± 0.03 per-cent, which was found to be increase significantly (P<0.05) up to 14th day, then slight decrease was found up to 30th days of observation.

The analysis of variance revealed significant ($P<0.05$) influence of *T. canis* infestation on blood TLC values in rabbits. The average estimate values of TLC on 2nd, 7th, 14th and 21st day were observed to be increase by 1.57, 1.76, 1.83 and 0.04 respectively than the control group while on day 30th of observation significant fall by 0.68 ($10^3/mm^3$) from control group was recorded (Table 6). However difference were non-significant among the estimated value of TLC on 2nd, 7th and 14th day but the difference was significant among the estimated value on 21st and 30th days of observation. The lowest average value of TLC recorded was (7.35%) on 30th days of observation.

Packed Cell Volume(PCV):

Mean ± S.E. with CV% of PCV at different days of interval have been presented in table-6 and in figure-3. The average estimated values of PCV in control group was recorded to be 34.05 ± 0.16 percent, which was found to be decreased significantly ($P<0.05$) in infected rabbits in subsequent days of interval up to 30th day of observation.

The analysis of variance revealed significant ($P<0.05$) influence of *T. canis* infestation on blood PCV values in rabbits. The average values of PCV on 2nd, 7th, 14th, 21st and 30 day were observed to be decreased by 0.32, 0.44, 0.54, 0.91 and 0.93 respectively than the control (Table 6). However differences were non-significant among 2nd, 7th and 14th and also between 21st and 30th day (table 6). The lowest average

estimate of PCV (33.12%) was recorded on 30th day of post inoculation of infection.

Differential leucocyte count (DLC):

Original mean values of various leucocyte and average of corresponding arc sin ± S.E. along with CV% are presented in Table-7

Lymphocytes: Mean its corresponding arc-sin value ± S.E. with CV% at different days of interval have been presented in table 7 and in the figure 4. The average estimate of Hb in control group was estimated to be 46.26 ± 0.33 percent. Which was found to decrease significantly ($P<0.05$) in sub-sequent days of interval up to 14th days then significant increase was found up to 30th days of observation.

Analysis of variance revealed significant ($P<0.05$) influence of *T. canis* infestation on blood lymphocytes values in rabbit. The average estimated percentage of lymphocytes on 2nd, 7th, 14th, 21st and 30th day were observed to be decreased by 1.49, 5.98, 8.32, 7.38 and 2.87 respectively than the control group (Table 7). The lowest average value of lymphocyte recorded was (34.94%) on 14th days of observation.

Neutrophils : Mean arc sin value of neutrophil ± S.E. with CV% at different days of interval have been presented in table - 7 and in the fig 4.The average estimated value of neutrophils in control group was estimated to be 40.74± 0.29 per-cent and there was increase found on 2nd day of observation but the difference was statistically non-significant. However significant

decreased in values of neutrophil on subsequent days of interval up to 21st were noted and again significant rise in the values observed on 30th days of observation.

Analysis of variance revealed significant (P<0.05) influence of migration of *T. canis* on blood neutrophils counts in rabbit. The average number of neutrophil on 2nd, 7th, 14th, 21st and 30th day were observed to be increased/decreased by + 0.23, 2.57, 2.21, 2.10 and 0.69 respectively than the control group (Table 7). The lowest average estimate (38.17%) was recorded on 7th days of observation.

Eosinophils : Mean its corresponding arc-sin value ± S.E. with CV% at different days of interval have been presented in table-7 and in the fig 4. The average estimate of eosinophil in control group was recorded to be 7.5 ± 0.81 percent which was found to be increase significantly in subsequent days of interval up to 14th day then there was decrease found in the eosinophil values up to 30th days of observation.

Analysis of variance revealed significance (P<0.05) influence of *T. canis* larval migration on blood eosinophils values in rabbits. The average number of eosinophils on 2nd, 7th, 14th, 21st and 30th day were observed to be increased by 3.65, 16.33, 19.16, 17.7 and 8.68 respectively than the control(Table 7). The lowest average value of eosinophil was recorded (7.54%) on 2nd days of observation.

Monocyte : Mean its corresponding arc-sin value ± S.E. with CV% at different days of interval have been presented in table-

7 and in the fig 4. The average estimated value of monocyte in control group was recorded to be 9.92 ± 0.53 percent and on 2nd day observation the estimated value of monocytes % was recorded to be 10.23 ± 0.63, but difference between these two values was statistically non-significant. There was no change observed on day 7th value from previous observation. However in later days of observation, i.e. 14th and 21st days, there was fall in monocytosis but this difference was analysed non-significant while rise in estimated value on 30th day (10.23%) was also varied non-significantly from previous days of observation and control group. No significant change observed throughout the period of observations from control group.

Table – 1 : Prevalence of toxocariosis in bovine calve and Pup population of Patna and its surrounding area.

Name of animals	No. of sample examined	No. of sample found positive	Percentage of infection
Calves	169	136	80.47
Pups	147	62	42.17
Total	316	198	62.65

Table – 2 : Effect of sex on *Toxocara vitulorum* (adult worm) recovered from slaughtered buffalo claves (From Patna abattoir).

Five samples of abattoir material	Male	Female	χ^2_{idf}
Total No. of worm recovered	37	32	0.362^{NS}
Average no. of worm/calve	7.40	6.40	

NS = Non-significant.

Table – 3 : Effect of sex on *Toxocara canis* (adult worm) recovered from intestine of sacrificed stray pups.

Sacrificed Five pups	Male	Female	χ^2_{idf}
Total No. of worm recovered	25	23	0.08^{NS}
Average no. of worm/pup	5	4.6	

NS = Non-significant.

Table – 4 : Mean ± S.E. along with CV% of blood parameters in rabbits of control and infected group with *Toxocara vitulorum* on different days of observation.

Days	Hb (g%) Mean ± S.E.	TLC ($10^3/mm^3$) Mean ± S.E.	PCV (%) Mean ± S.E.
0 days (control)	$11.32^{d} \pm 0.05$ (1.14)	$8.03^{a} \pm 0.03$ (0.90)	$34.05^{b} \pm 0.16$ (1.08)
2nd	$10.76^{c} \pm 0.05$ (1.11)	$9.78^{b} \pm 0.02$ (0.51)	$33.87^{b} \pm 0.02$ (0.14)
7th	$10.70^{c} \pm 0.04$ (0.93)	$11.60^{d} \pm 0.02$ (0.43)	$33.94^{b} \pm 0.02$ (0.14)
14th	$10.16^{b} \pm 0.05$ (1.08)	$14.27^{f} \pm 0.02$ (0.35)	$33.31^{a} \pm 0.07$ (0.51)
21st	$9.80^{a} \pm 0.04$ (1.02)	$13.17^{e} \pm 0.04$ (0.68)	$32.52^{c} \pm 0.14$ (0.95)
30th	$10.82^{c} \pm 0.03$ (0.76)	$11.06^{c} \pm 0.03$ (0.72)	$33.10^{a} \pm 0.04$ (0.27)

Values with different super scripts (column-wise) differed significantly ($P<0.05$).

Values within the parenthesis are CV%.

1. : Figure showing mean of blood parameters in rabbits of control and infected groups with *Toxocara vitulorum on different days of observation*.

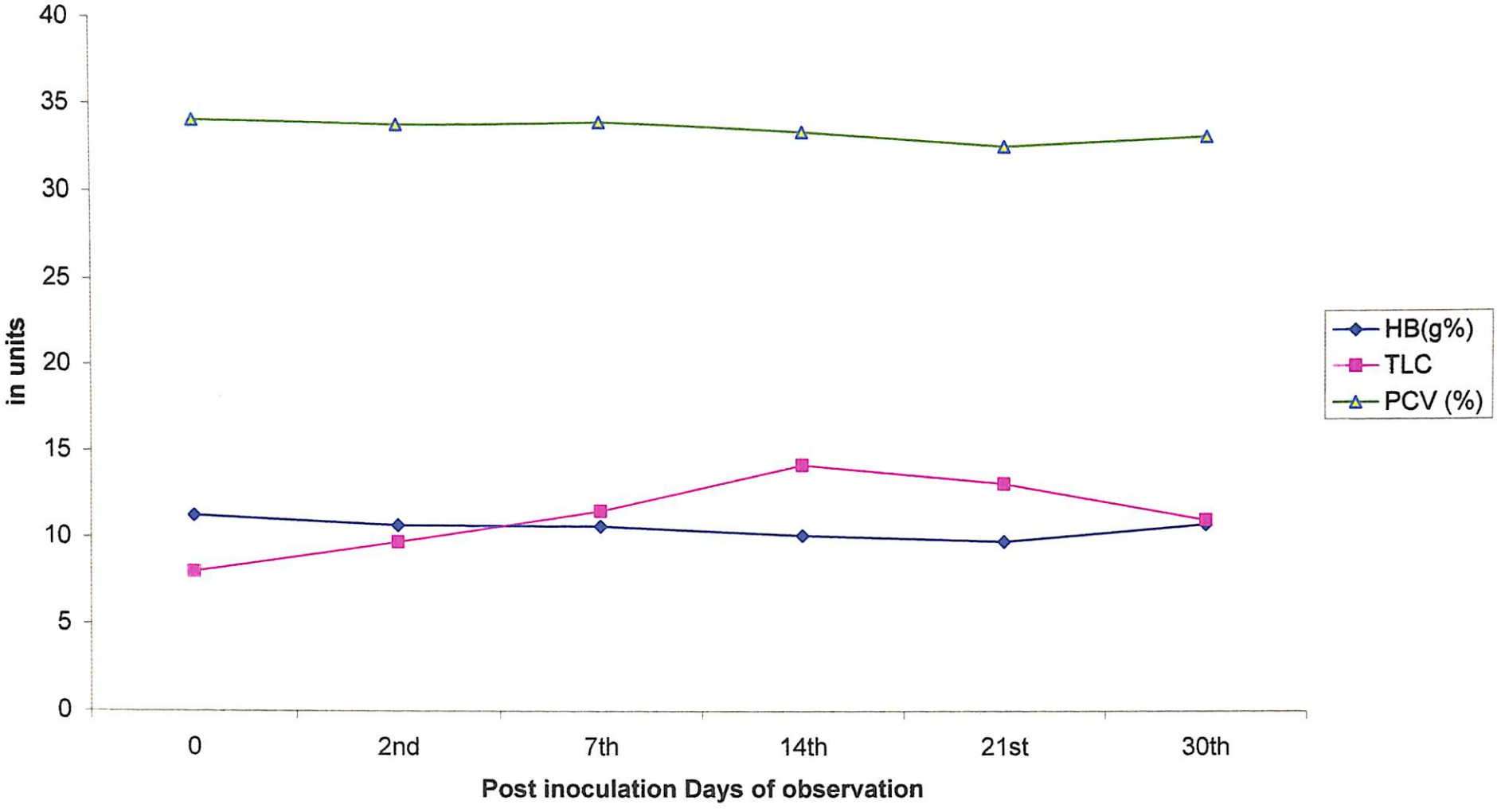


Table – 5 : Mean its corresponding arc-sin values ± S.E along with CV% of DLC of rabbits in control and infected group with *Toxocara vitulorum* on different days of observation.

Days	Lymphocyte (%) Mean ± S.E.	CV%	Neutrophil (%) Mean ± S.E.	CV%	Eosinophil (%) Mean ± S.E.	CV%	Monocyte (%) Mean ± S.E.	CV%
(control) 0 days	$46.26^{f} \pm 0.33$ (52.18)	1.59	$40.74^{a} \pm 0.29$ (42.58)	1.59	$7.54^{a} \pm 0.81$ (1.64)	24.00	$9.92^{a} \pm 0.53$ (2.93)	12.00
2nd	$44.31^{e} \pm 0.21$ (48.79)	1.08	$41.20^{e} \pm 0.22$ (43.39)	1.23	$12.30^{b} \pm 0.71$ (4.47)	13.00	$9.55^{a} \pm 0.64$ (2.70)	15.07
7th	$40.86^{c} \pm 0.21$ (42.79)	1.17	$37.22^{a} \pm 0.30$ (36.58)	1.82	$24.49^{d} \pm 0.28$ (17.18)	2.57	$10.23^{a} \pm 0.63$ (3.10)	13.78
14th	$38.17^{a} \pm 0.21$ (38.19)	1.28	$37.46^{a} \pm 0.26$ (36.98)	1.57	$27.40^{e} \pm 0.41$ (21.16)	3.35	$10.23^{a} \pm 0.63$ (3.10)	13.78
21st	$38.64^{b} \pm 0.18$ (38.99)	1.06	$39.35^{b} \pm 0.21$ (40.19)	1.24	$24.64^{d} \pm 0.30$ (17.38)	2.71	$9.92^{a} \pm 0.53$ (2.93)	12.09
30th	$43.16^{d} \pm 0.21$ (46.79)	1.08	$40.28^{c} \pm 0.21$ (41.70)	1.19	$15.96^{c} \pm 0.55$ (7.52)	7.76	$10.23^{a} \pm 0.63$ (3.10)	13.78

Values with different super scripts (column-wise) differed significantly ($P<0.05$).
Values within the parenthesis are geometric mean.

2. : Figure showing mean of arc-sin values of DLC of rabbits in control and infected groups with *Toxocara vitulorum* on different days of observation.

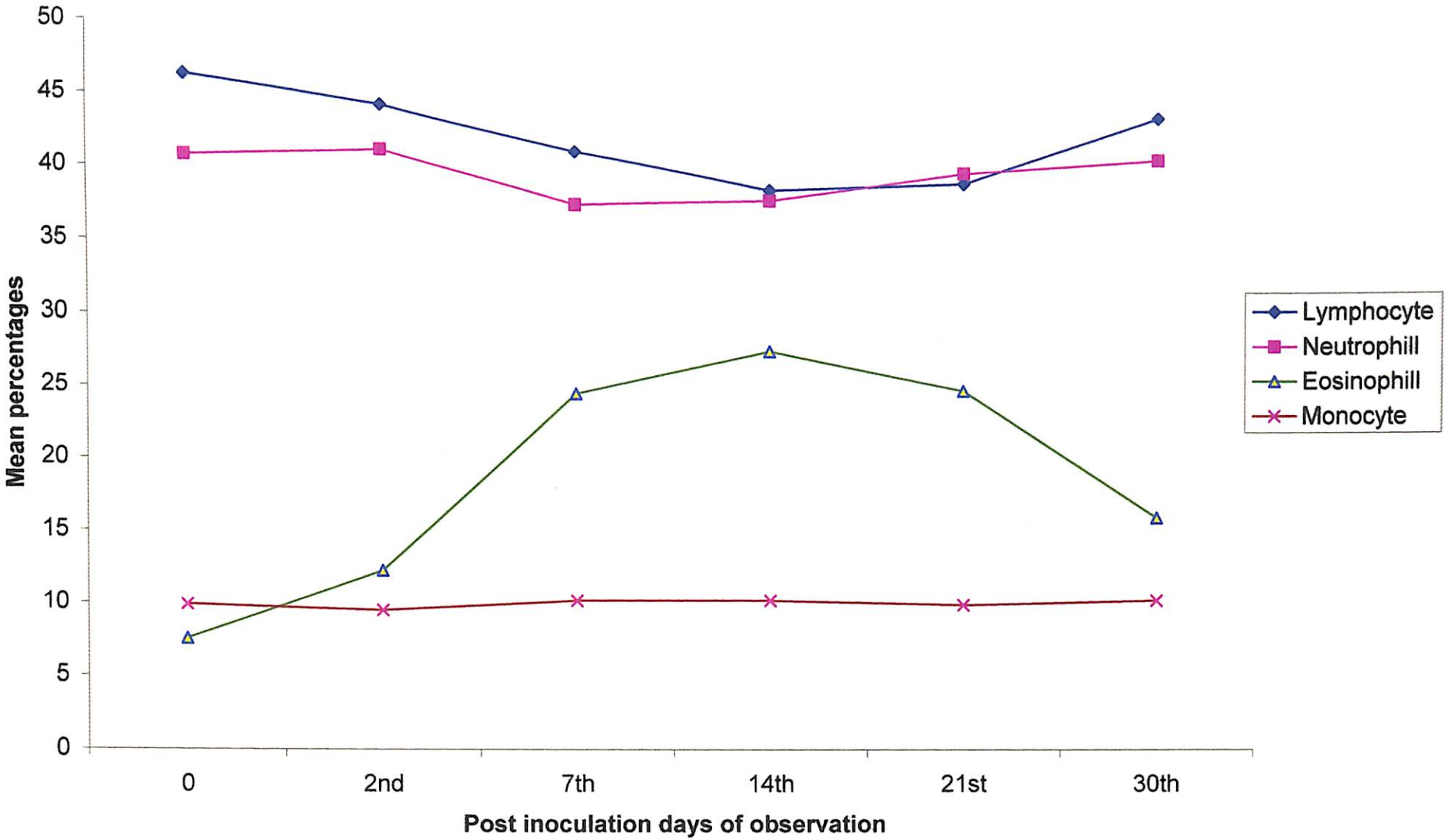


Table – 6 : Mean ± S.E. along with CV% of blood parameters in rabbits of control and infected groups with *Toxocara canis* on different days of observation.

Days	Hb (g%) Mean ± S.E.	TLC ($10^3/mm^3$) Mean ± S.E.	PCV (%) Mean ± S.E.
(control) 0 days	$11.32^{f} \pm 0.05$ (1.14)	$8.03^{b} \pm 0.03$ (0.90)	$34.05^{c} \pm 0.16$ (1.08)
2nd	$9.68^{d} \pm 0.05$ (1.34)	$9.60^{c} \pm 0.23$ (5.52)	$33.73^{b} \pm 0.03$ (0.20)
7th	$8.86^{b} \pm 0.04$ (1.24)	$9.79^{c} \pm 0.32$ (7.35)	$33.61^{b} \pm 0.08$ (0.05)
14th	$8.08^{d} \pm 0.03$ (0.99)	$9.86^{c} \pm 0.21$ (6.08)	$33.51^{b} \pm 0.11$ (0.77)
21st	$9.48^{c} \pm 0.03$ (0.84)	$8.17^{b} \pm 0.01$ (2.93)	$33.14^{a} \pm 0.03$ (0.24)
30th	$10.68^{e} \pm 0.05$ (1.21)	$7.35^{a} \pm 0.02$ (0.81)	$33.12^{a} \pm 0.04$ (0.30)

Values with different super scripts (column-wise) differed significantly ($P<0.05$).

Values within the parenthesis are CV%.

3. : Figure showing mean of blood parameters in rabbits of control and infected groups with *Toxocara canis* on different days of observation.

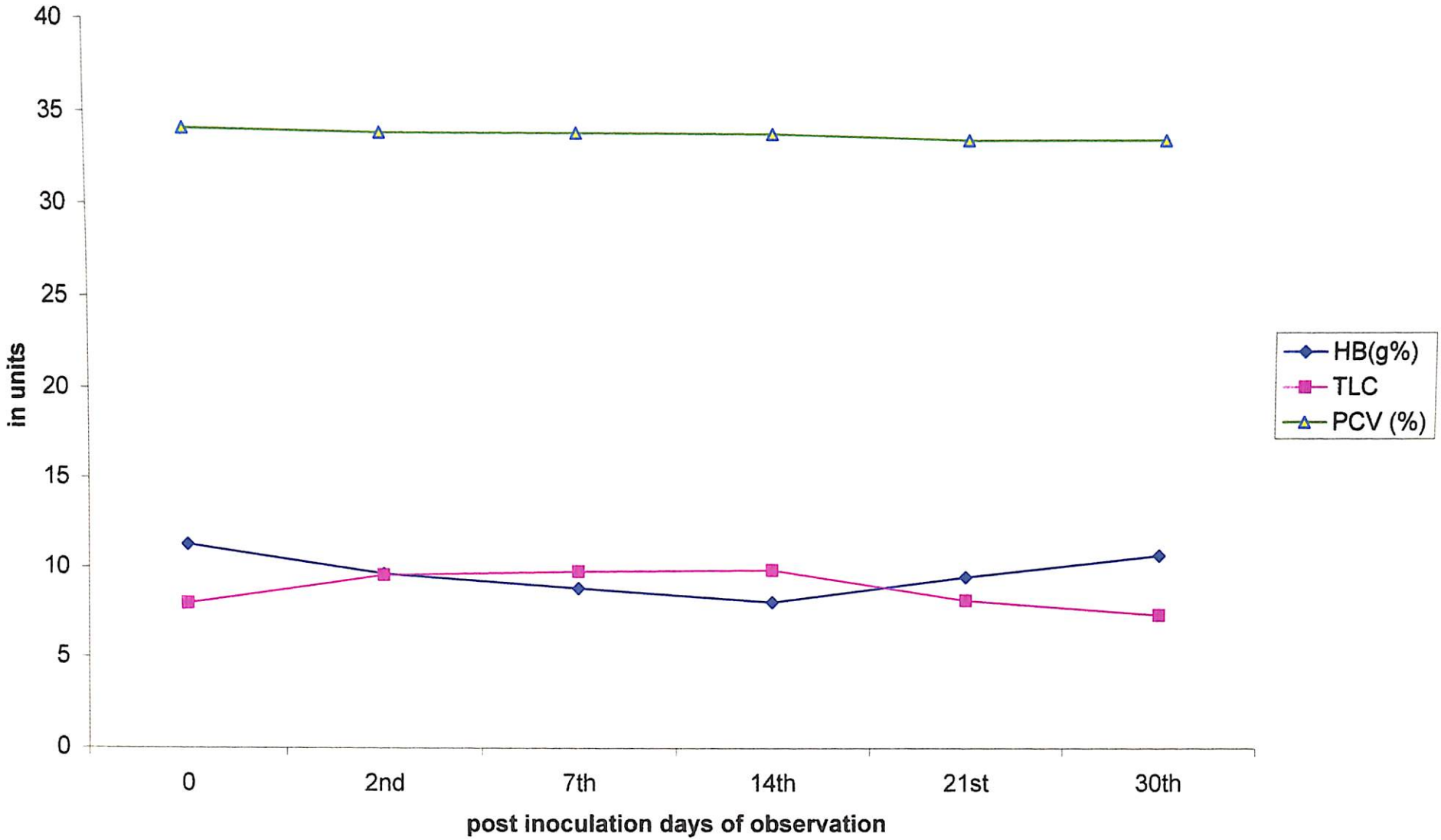


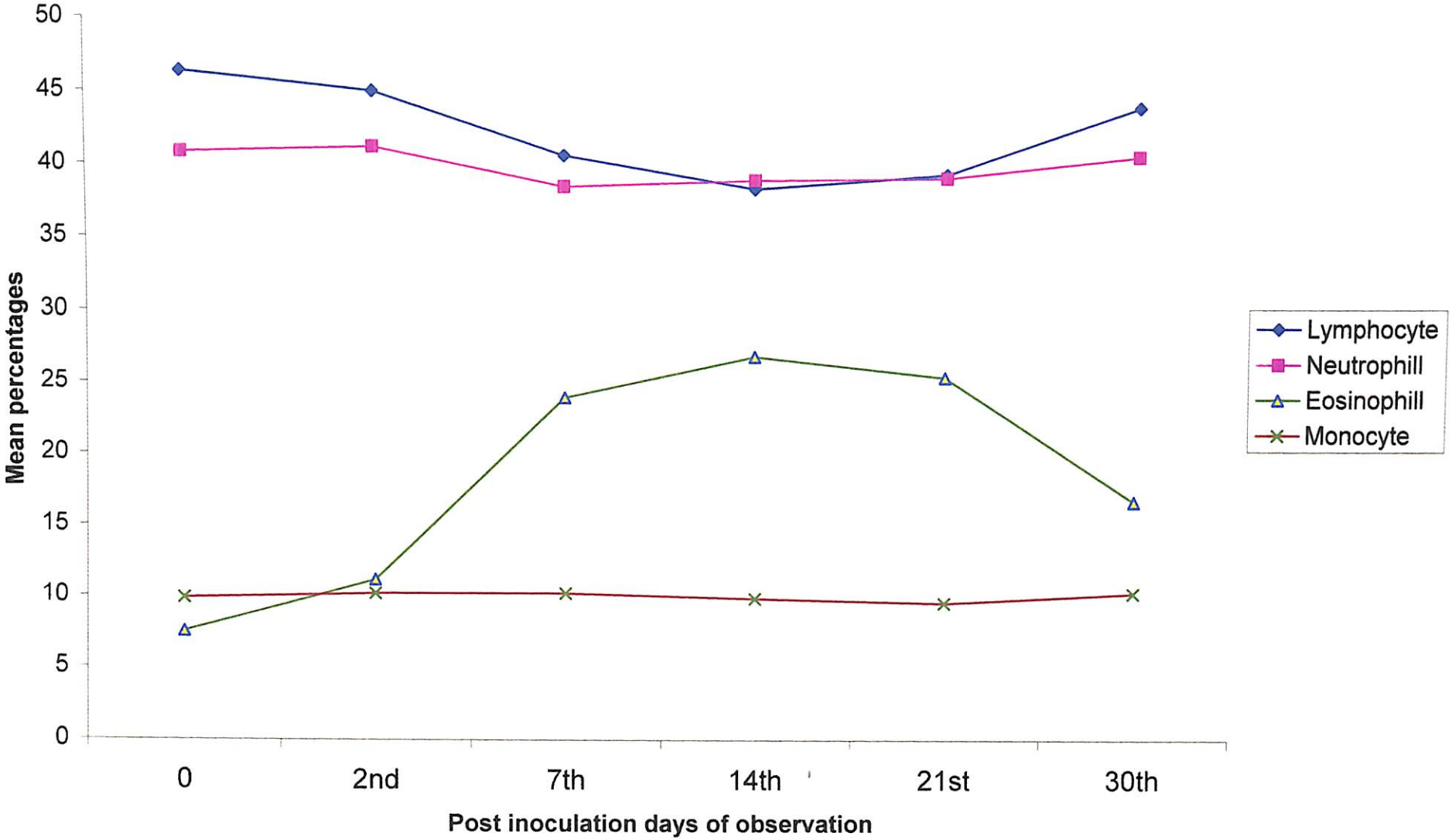
Table – 7 : Mean its corresponding arc-sin values ± S.E along with CV% of DLC of rabbits in control and infected group with *Toxocara canis* on different days of observation.

Days	Lymphocyte (%) Mean ± S.E.	CV%	Neutrophil (%) Mean ± S.E.	CV%	Eosinophil (%) Mean ± S.E.	CV%	Monocyte (%) Mean ± S.E.	CV%
(control) 0 days	$46.26^{f} \pm 0.33$ (52.18)	1.59	$40.74^{b} \pm 0.29$ (42.58)	1.54	$7.54^{a} \pm 0.81$ (1.64)	24.00	$9.92^{a} \pm 0.53$ (2.93)	12.00
2nd	$44.77^{e} \pm 0.29$ (49.58)	1.45	$40.97^{c} \pm 0.40$ (43.18)	2.22	$11.19^{b} \pm 0.55$ (3.72)	11.08	$10.23^{a} \pm 0.63$ (3.10)	13.78
7th	$40.28^{c} \pm 0.21$ (41.79)	1.19	$38.17^{a} \pm 0.21$ (38.19)	1.28	$23.87^{d} \pm 0.39$ (16.36)	3.64	$10.23^{a} \pm 0.63$ (3.10)	13.78
14th	$37.94^{a} \pm 0.21$ (37.79)	1.29	$38.53^{a} \pm 0.21$ (38.79)	1.27	$26.70^{f} \pm 0.26$ (20.18)	2.24	$9.86^{a} \pm 0.76$ (2.86)	17.24
21st	$38.88^{b} \pm 0.29$ (39.38)	1.69	$38.64^{a} \pm 0.31$ (38.98)	1.83	$25.24^{e} \pm 0.27$ (18.18)	2.45	$9.55^{a} \pm 0.64$ (2.70)	15.07
30th	$43.39^{d} \pm 0.21$ (47.19)	1.08	$40.05^{b} \pm 0.29$ (41.38)	1.64	$16.62^{c} \pm 0.39$ (8.16)	5.29	$10.23^{a} \pm 0.63$ (3.10)	13.78

Values with different super scripts (column-wise) differed significantly ($P<0.05$).

Values within the parenthesis are geometric mean.

4. : Figure showing mean of arc-sin values of DLC of rabbits in control and infected groups with *Toxocara canis* on different days of observation.



LARVAL RECOVERY ON EXPERIMENTAL INOCULATION OF INFECTION OF *TOXOCARA VITULORUM* IN DIFFERENT ORGANS.

Single dose of 5000 embryonated eggs of *Toxocara vitulorum* was inoculated in 25 rabbits and larval migration was assessed on the basis of number of larval recovery in various organs on 2nd, 7th, 14th, 21st and 30th days of post inoculation. Mean ± S.E. along with CV% of larval recovery in liver, lung kidney spleen, Muscles and Intestinal content are presented in Table-8.

LIVER :

It is evident from the table-8 that mean larval recovery in liver of rabbit ranged between 179.2 to 367.8 during whole period of observation. The data clearly indicated the declining trend in number of larval recovery with advancement of post inoculation days, as maximum number of larvae found on 2nd day (367.8) of observation followed by 7th (302.4), 14th (262.4) and 21st (218.4) day while minimum number of larvae was detected on 30th (179.2) day of observation. Statistical analysis revealed significant influence of *Toxocara vitulorum* larval migration in liver on different days, as analysis of varience indicated significant ($P<0.05$) difference in larval counts in all observation days.

LUNGS :

Mean ± S.E. with CV% of larval count in lung at 2nd, 7th, 14th, 21st and 30th days of observations depicted in the table-8

showed significant (P<0.05) increase in larval count from 2nd days (74.8) to 7th (95.0) post inoculation there after a gradual decrease recorded in larval count on 14th, 21st and 30th observation days by 19.0, 41.6, 52.8 respectively than the 7th days of post observation. The average larval count in lungs, on all observation days differ significantly (P<0.05) however the difference were non-significant among larvae on 2nd and 14th days of observation.

KIDENY & SPLEEN :

In present investigation any larvae was not detected in all days of observation in both the organs.

SKELETAL MUSCLE :

Skeletal muscle was also examined for the presence of larvae of *T. Vitulorum* in different days of observation but absence of larvae noted up to 21st day of observation while on day 30 post infection mean number larval count was 34.6(Table 8).

INTESTINAL CONTENT :

During examination on intestinal content of rabbit on different days of post inoculation it was observed that mean number of larval counts were 3.4, 21.4 and 5.0 on second, 7th and 14th days of observations. Statistical analysis revealed that larval count increased significantly (P<0.05) from 2nd to 7th days observation and decreased significantly (P<0.05) on 14th day while larval count was nil on 21st and 30th days of observation (Table 8).

Data pertaining to average larvae recovered in various days of observation depicted, that maximum larval count was detected on 2nd day (446.4) of observation and gradually it declined in 7th, 14th, 21st and 30th days, as number of larvae recovered were 419, 341.8, 271.8 and 256.0 respectively (Table 8). However analysis of variance revealed significant differences ($P<0.05$) on all the days of observation.

LARVAL RECOVERY ON EXPERIMENTAL INOCULATION OF INFECTION OF *TOXOCARA CANIS* IN DIFFERENT ORGANS:

Single dose of 5000 embryonated eggs of *T. canis* administered in 25 rabbits and migratory behaviour of *T. canis* larvae was assessed on the basis of average larval count in different organs of 5 rabbits on 2nd, 7th, 14th, 21st and 30th days of post infection. Mean ± S.E. along with CV% of larval count in liver lungs, kidneys, spleen, skeletal muscle and Intestinal content are presented in table-9.

LIVER :

During examination of liver samples, maximum number of larvae recovered on 2nd day of migration (377.6) then a declining trend in larval count was observed with the advancement of post inoculation days. The gradual decrease pertaining the larval count from second day was 71.0, 106.6, 149.0, 189.2 on 7th, 14th, 21st and 30th days of observation respectively(Table 9). However, analysis of variance revealed significant ($P<0.05$) difference between all the days of larval count.

LUNGS :

The mean larval count estimated in lungs indicated increasing trend in recovered larvae up to 7th day observation (95.2) from 2nd day (79.6). There was gradual decrease recorded from peak value, on later days of observation as differences were noted as 19.4, 33.8 and 51.8 on 14th, 21st and 30th days respectively (Table 9). While analysis of variance revealed significant differences ($P<0.05$) in larval count of all the days of observation.

KIDNEY & SPLEEN :

In present investigation migration of larvae in kidney and spleen was not observed, as there was total absence of larvae detected throughout the period of observation.

SKELETAL MUSCLES :

The *T. canis* larval migration was delayed as no larvae recovered from the skeletal muscles in earlier observations up to 14th days of post inoculation of infection. Later on 21st day mean larval count was 16.20 in muscles, which significantly increased ($P<0.05$) on 30th days (35.4) of observation (Table 9).

INTESTINAL CONTENT :

Transmission of larvae was evident in intestine on 7th day of post-inoculation as mean number 24.2 larvae recovered from intestinal content but there was a significant fall ($P<0.05$) noted in larval count of 14th day (6.4) observation while on later days of observation complete absence of larvae found in intestinal content of infected rabbits with *T. canis*(Table 9).

Mean number of overall larval count depicted in table-9 revealed that maximum number of *T. canis* larvae invaded the liver tissues followed by lungs in present trial. Considerable larval migration was also evident in intestinal content and skeletal muscles but was, in lesser extent to previous results, while evidence of larval migration was not observed in spleen or kidney in present trial. Overall mean number of larval count of different days of interval estimated to have significant variation ($P<0.05$) in all days of observation. Larval migration on day 2^{nd} was maximum (457.4) which subsequently decreased by 31.4, 104.2, 151.2, 190.2 respectively on 7^{th}, 14^{th}, 21^{st} and 30^{th} days of observation (Table 9).

Table – 8 : Mean ± S.E. along with CV% of larvae recovered from different organs and intestinal content of rabbit infected with *Toxocara vitulorum*.

Post infection days	No. of rabbit sacrificed	Liver	Lung	Kidney	Spleen	Skeletol muscles	Intestinal content	Total larvae recovered
2nd	5	$367.8^{e} \pm 2.64$ (1.60)	$74.8^{c} \pm 1.24$ (3.70)	–	–	–	$3.4^{a} \pm 0.74$ (49.11)	$446.4^{e} \pm 3.71$ (1.85)
7th	5	$302.4^{d} \pm 2.66$ (1.96)	$95.0^{d} \pm 1.0$ (2.34)	–	–	–	$21.4^{b} \pm 0.92$ (9.67)	$419^{d} \pm 2.43$ (1.29)
14th	5	$262.4^{c} \pm 2.27$ (1.93)	$74.2^{c} \pm 0.86$ (2.58)	–	–	–	$5.0^{a} \pm 0.70$ (31.6)	$341.8^{c} \pm 1.53$ (1.00)
21st	5	$218.4^{b} \pm 1.78$ (1.81)	$53.4^{b} \pm 0.92$ (3.87)	–	–	–	–	$271.8^{b} \pm 1.49$ (1.22)
30th	5	$179.2^{a} \pm 0.86$ (1.07)	$42.2^{a} \pm 0.86$ (4.54)	–	–	34.6 ± 1.21 (7.80)	–	$256.0^{a} \pm 1.30$ (1.13)

Values with different super scripts (column-wise) differed significantly ($P<0.05$).
Values within the parenthesis are CV%.

5. : Figure showing mean of larvae recovered from different organs and intestinal content of rabbit infected with *Toxocara vitulorum* .

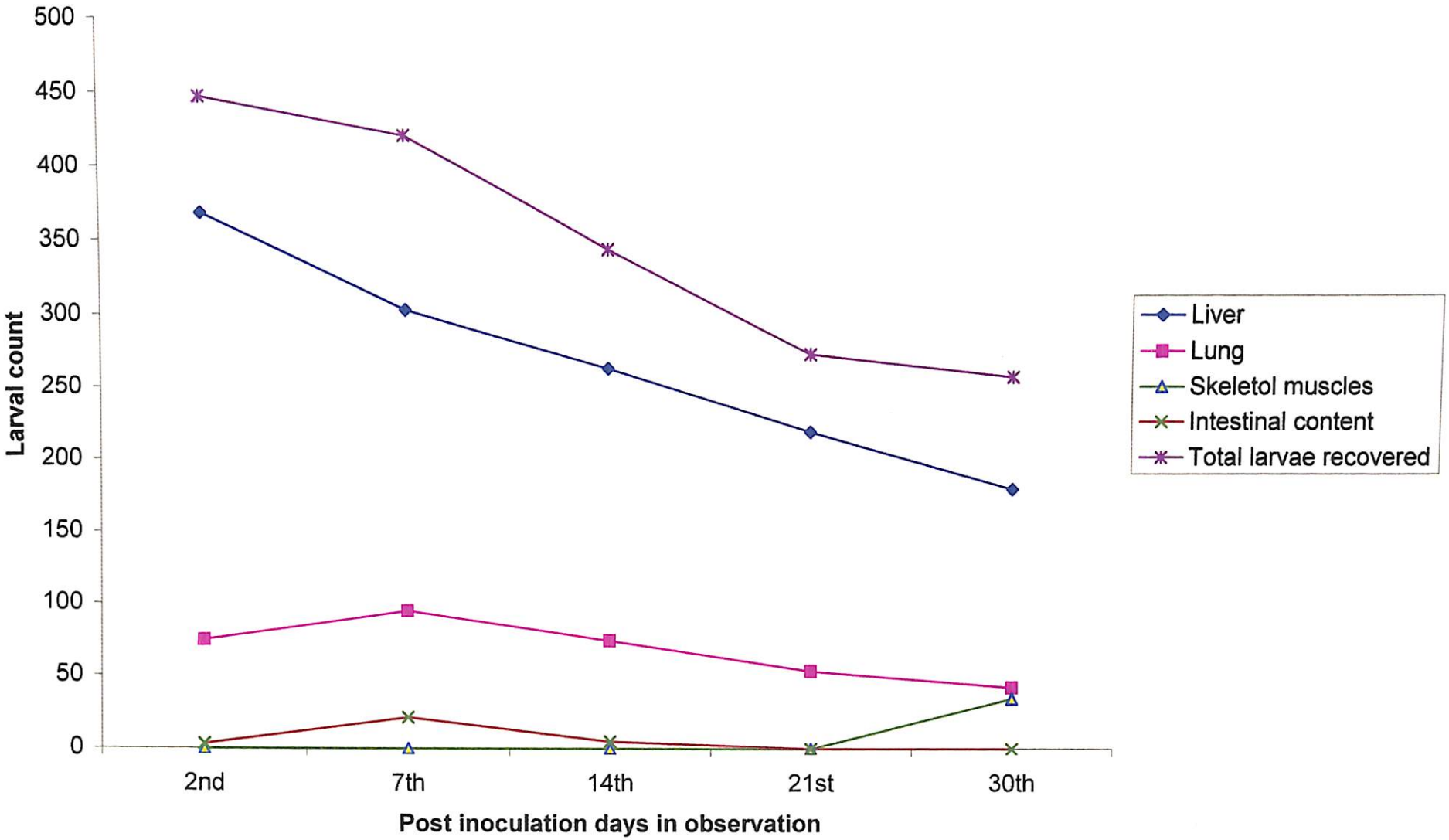
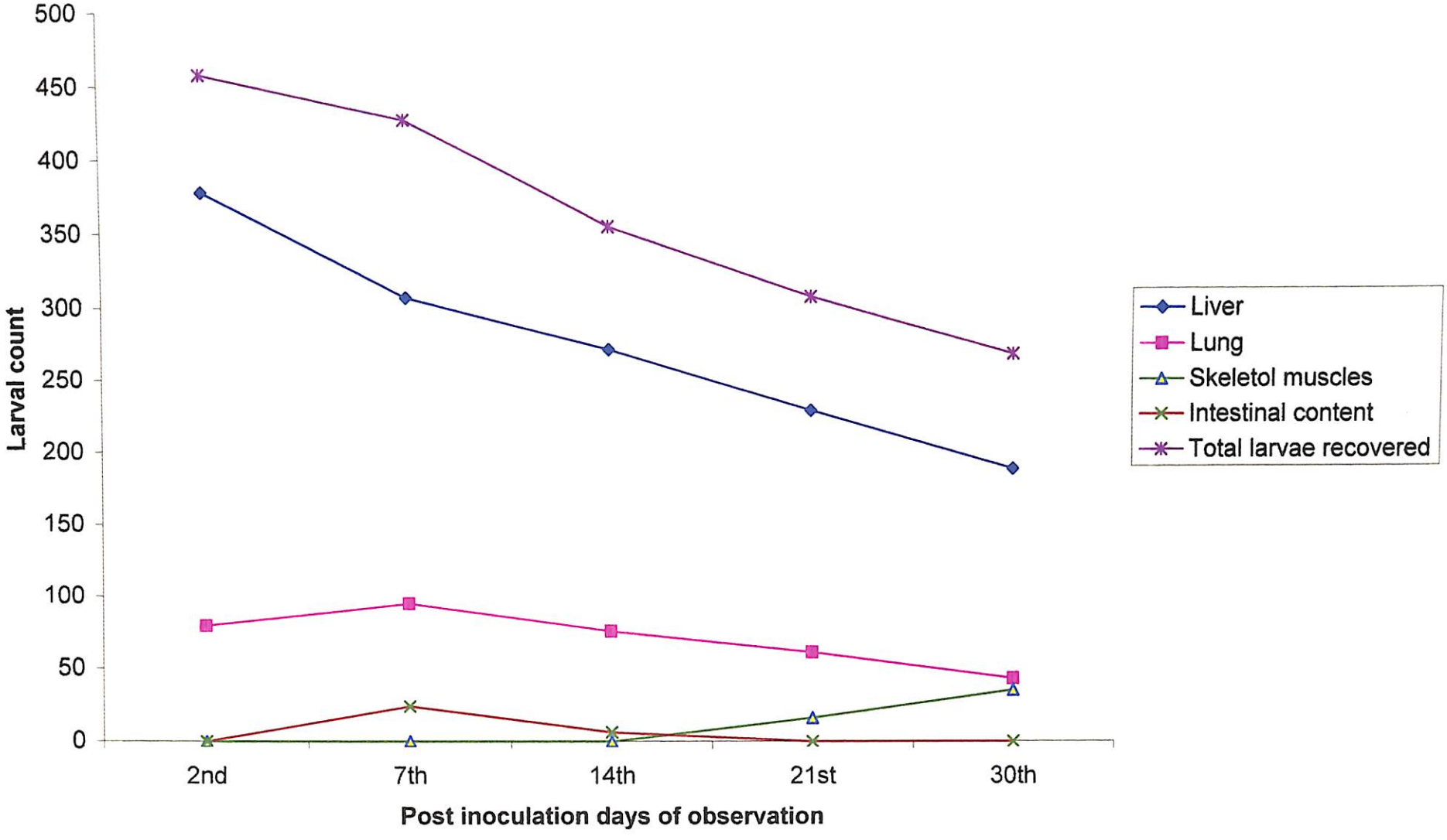


Table – 9 : Mean ± S.E. along with CV% of larvae recovered from different body organs and intestinal content of rabbit infected with *Toxocara canis*.

Post infection days	No. of rabbit sacrificed	Liver	Lung	Kidney	Spleen	Skeletol muscles	Intestinal content	Total larvae recovered
2nd	5	$377.6^{e} \pm 1.53$ (0.90)	$79.6^{d} \pm 1.29$ (3.61)		–	–	–	$457.4^{e} \pm 2.01$ (0.98)
7th	5	$306.6^{d} \pm 1.43$ (1.04)	$95.2^{e} \pm 1.02$ (2.5)	–	–	–	$24.2^{a} \pm 1.46$ (13.51)	$426^{d} \pm 2.30$ (1.20)
14th	5	$271^{c} \pm 1.30$ (1.07)	$75.8^{c} \pm 1.15$ (3.40)	–	–	–	$6.4^{b} \pm 1.03$ (35.93)	$353.2^{c} \pm 2.82$ (1.78)
21st	5	$228.6^{b} \pm 2.66$ (2.59)	$61.4^{b} \pm 1.29$ (4.69)	–	–	$16.20^{b} \pm 2.42$ (3.33)	–	$306.2^{b} \pm 2.65$ (1.93)
30th	5	$188.4^{a} \pm 1.60$ (1.89)	$43.4^{a} \pm 0.92$ (4.75)	–	–	$35.40^{a} \pm 1.91$ (12.06)	–	$267.2^{a} \pm 3.88$ (3.24)

Values with different super scripts (column-wise) differed significantly ($P<0.05$).
Values within the parenthesis are CV%.

6. : Figure showing mean of larvae recovered from different organs and intestinal content of rabbit infected with *Toxocara canis*.



STUDIES ON GROSS AND HISTOPATHOLOGICAL CHANGES IN VARIOUS ORGANS OF RABBITS DUE TO MIGRATION OF *TOXOCARA VITULORUM* LARVAE IN VARIOUS DAYS OF POST-INFECTION

LIVER :

Grossly, liver surfaces showed multiple white spots on day 2nd p.i. and pale coloured nodules were found on the surface on 7th day. While on 14th and 21st day p.i. considerable reduction of spots was seen which finally disappeared on day 30 p.i. However enlargement in the size of liver was evident throughout the experimental period and cut portion of liver were found bulged with heavy larval load on day 2nd, 7th and 14th of observation. Numerous larvae were also found lodged on surface of liver on above mentioned periods of observation.

Microsopically, cellular changes in liver were of degenerative type, and structure of liver cells found altered on 2nd, 7th, 14th and 21st days with congestion were observed. (Figure-26) Hepatic vein was dilated due to extensive histotropic larval migration of *T. vitulorum* throughout the periods of observation. Extensive haemorrhage and necrosis along with infiltration of eosinophils and macrophages were also viewed on all the days of observation however on day 30 p.i. these reactions were found in lesser extent.

LUNGS :

Macroscopically, severe congestion in lungs was observed up to day 21 p.i. On incision yellowish foamy exudate oozed

haemorrhagic spots and on day 21st and 30th, peticheal haemorrhagic spot were seen on surface of kidney.

Histopathologically also, there was no structural changes found in the kidney sections on day 2nd p.i but on day 7th and 14th p.i majority of cortex and medullary blood vessels were dilated Congestion in vessels was also observed due to infiltration of erythrocytes. Interstitial tissue, cut of cortex portion showed presence of larvae on day 21st and 30 p.i infiltration of inflammatory cells comprising mononuclear cells in the interlobular septa region was obvious. The degenerative changes were found in lining epithelium of tubules (figure-31).

SPLEEN :

Grossly any anamolies was not detected up to day seven observation where as slight enlargement in spleen was found on day 14th observation. On day 21st slight spleenomegaly was evident along with very mild congestion. On day 30th p.i. complete resolutions in changes were observed and spleen was found absolutely normal.

Microscopically at day 2nd p.i. any abnormal changes did not account in cut section of spleens. But on day 7th, 14th and 21st vascular congestion and haemorrhagic foci were visualized. At day 21st and 30th p.i. sinusoid were dilated and there were haemorrhagic tracts with or without larvae surround by macrophages and giant cells. Cells of malpighian tubules were found hypertrophied.

INTESTINE :

Grossly, on day 2 and 7 p.i. the examination of cut portions of various port of intestine revealed minute haemorrhagic spots while in later days of observation rather big haemorrhagic spots were visualized. However on day 30 p.i. there was absolute resolution of haemorrhagic spots but blood mixed faecal materials was observed in lumen of the large intestine.

Microsopically cut sections of intestine showed degeneration and desquamation of mucous membrane on day 2nd p.i. while on 7th and 14th p.i. there was lack of differentiation of parietal cells along with rapid cell division resulting marked hyperplasia. Inflammation was also noticed in sub mucosal areas with infiltration of mononuclear cells along with large number of cut sections of larvae on day 21 p.i. on day 30 p.i., showed almost normal histological structures in intestine.

SKELETAL MUSCLES :

Grossly no structural changes were observed in the strature of skeletal muscles of rabbits infected with *T. vitulorum* at all the days of observation of post infection.

However microscopically changes were not evident on day 2 p.i. in the cut section of muscles of rabbits infected with *T. vitulorum*, while on day 7 p.i. few migratory tracts were seen surrounded by macrophages and eosinophils, where as infiltration of lymphocytes were found in some migratory tracts

on 14th and 21st day of p.i. On day 30 p.i. reflected few granuloma, replacing the migratory routes. These granuloma were primarily consisted of granulation cells, macrophages and lymphocytes.

STUDIES ON GROSS AND HISTOPATHOLOGICAL CHANGES IN VARIOUS ORGANS OF RABBITS DUE TO MIGRATION OF *TOXOCARA CANIS* LARVAE ON VARIOUS DAYS OF POST-INFECTION :

LIVER :

Grossly on day 2nd post infection (p.i.) the size of liver was enlarged as compared to uninfected liver samples of the rabbit. Numerous larvae were found lodged superficially and many yellowish spots were seen at the site of larval attachment whereas day 7th p.i. cut portions of liver were bulged and presence of larvae was evident as pale elevated spots were observed on 7th p.i. days, on 14th day post infection less number of larvae were recovered, however yellow spots turned into brownish spots sized 1-2 mm along with congestion in liver. On day 21 p.i., gross changes revealed that hepatomegaly disappeared and number of brown spots were very less and day 30 p.i. liver was almost normal in shape and texture. Spots or nodules due to larval migration were less or disappeared.

Histological examination of cut sections of liver of infected rabbits revealed conspicuous migratory routes filled with haemorrhagic contents up to 7 days post infection (Figure 25).

Marked disorganization of liver cell structure was seen. Fatty degeneration with hepatostatosis was evident up to days 21, p.i. Haemorrhagic necrotic foci infiltrated with neutrophils and eosinophils were profound from 7th to 14th day p.i. and less prominent on 21st and 30th day observation. Presence of larvae was detected in liver parenchyma from day 2nd to day 14th. However no larvae could be found on 21st and 30th day, p.i. but degenerative changes along with infiltration of macrophages was clearly observed on both observation days.

LUNGS :

Grossly congestion was observed in lungs of infected rabbits with *T. canis*. Macroscopic changes in lungs were represented by various yellowish foci along with haemorrhagic lesions in sub pleural region from day 2 to day 14 p.i. The lungs architecture was found to be disorganized due to heavy larval burden and atelectasis, emphysema was also seen. Afterward on day 21 and 30 p.i. observation revealed decrease in congestion and resorption of the haemorrhagic lesions.

Microscopic changes showed the existence of inflammatory reactions as infiltration of neutrophil, eosinophils and lymphocytes observed with advance days of post infection days up to 21st day evidence of thickening of alveolar septum, bronchioles and lesions of interstitial bronchopneumonia together with pulmonary edema rest compensatory emphysema was also seen in earlier phase of infection (figure-27). Congestion of alveolar capillaries and alveoli was also observed along with infiltration of

macrophages. On day 21 and 30 p.i. haemorrhagic migratory routes was mostly replaced by serofibrinous exudates.

KIDNEY :

Macroscopically very slight congestion was observed on the surface of kidney along with slight enlargement and pale appearance on day 7 to 14 p.i.

Microscopically there was lymphocytic infiltration in cortex on day 7 and 14 p.i. however granular degeneration of glomerular capillaries was observed on similar days of observation (figure 30). At 21st and 30th day p.i. kidney regained original structure and signs of organ's reactivity towards larval invasion was not seen at the end of the experiment.

SPLEEN :

Grossly enlargement in the size of spleen samples in infected group was noticed at 14th day post infection. However it was assessed that spleen was not the target tissue in the route of larval migration throughout the period of study.

While histopathologically also spleen sections did not show any variation in comparision to control up to 7th day p.i. but on day 14 p.i. vascular congestion and haemorrhagic spots were visible. On day 21 p.i. dialation in sinusoids was visualized and haemorrhagic spots infiltrated with macrophages and giant cells were seen with extincting migratory routes. On day 30 p.i. these haemorrhagic spots were seen replaced by hyalinised connective tissues.

INTESTINE :

Pinhead size haemorrhagic spots observed less on 2nd day p.i. whereas at day 7th and 14th day p.i. many such spots were noticed especially in ileum and caecum. However in later days of observation (21st and 30th day) p.i. there was no sign of such spot.

Histopathologically, the section of intestine was almost normal as compared to control group of rabbits throughout the observation period, however shortening of villi and inflammation were observed on 7th and 14th day p.i. in sections of intestine.

SKELETAL MUSCLE :

Any major macroscopic change in the skeletal muscles of infected rabbits did not occur throughout the period of present investigation however small numbers of larvae were found in skeletal musculature without any gross modification in muscle architecture.

Microscopically modification associated with certain migratory tracts, which were surrounded by eosinophils and macrophages in the later phase of experiment (21st and 30th) However these changes were accompanied with infiltration of serous exudates in the endomyecium, perimyecium and interfolicular connective tissue.

A few granuloma were also observed in recovered tissue from larval migration.

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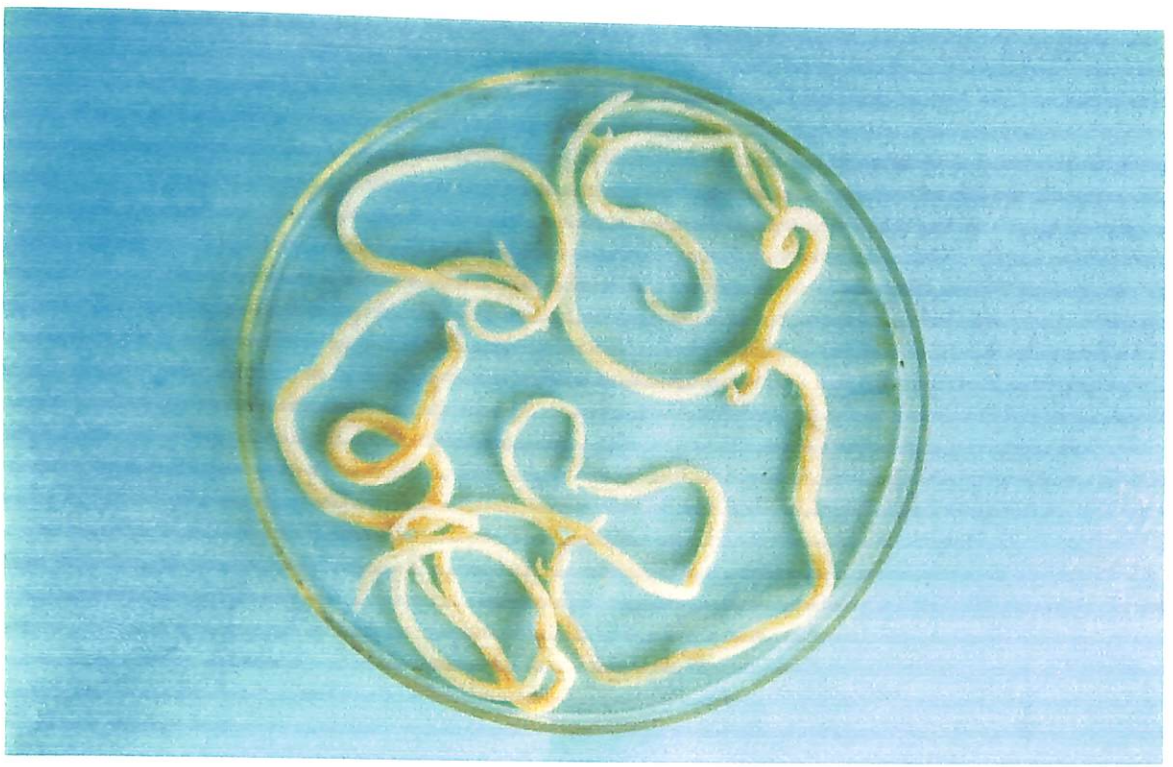
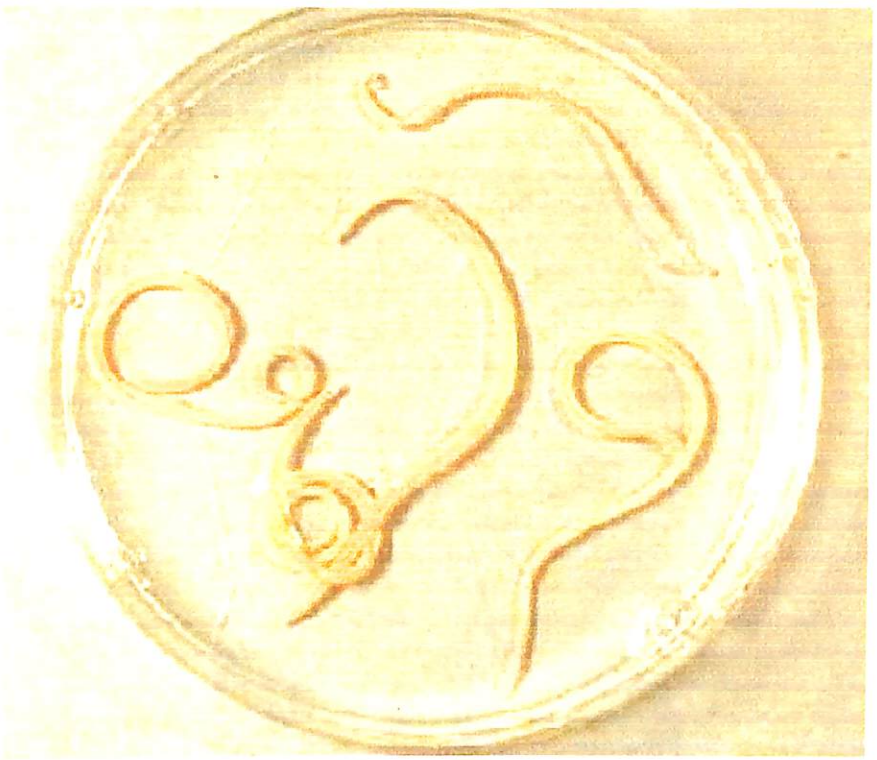
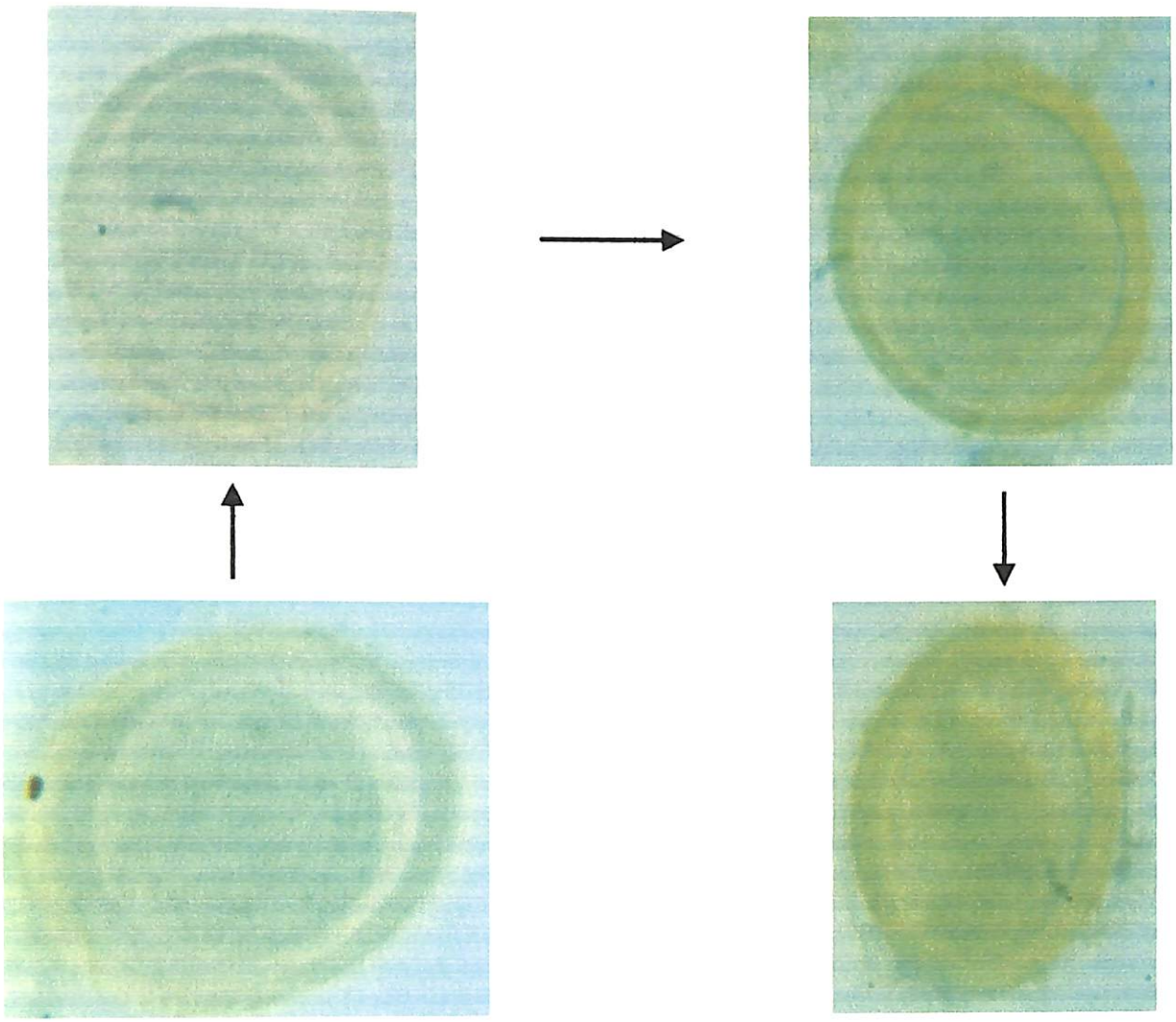


7. : Figure showing adult worms or *Toxocara vitulorum* collected from infected calves.



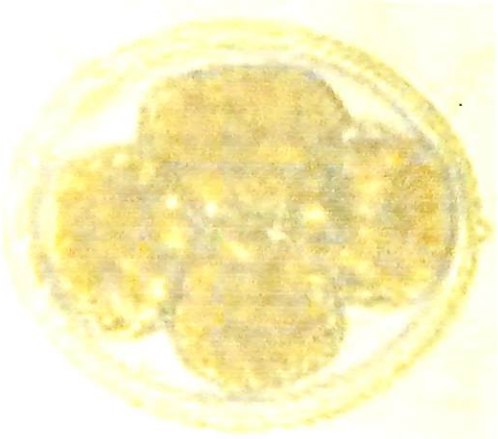
8. : Figure showing adult worms or *Toxocara canis* collected from infected pups.

9. : Figures showing sequential development of *Toxocara vitulorum* egg.

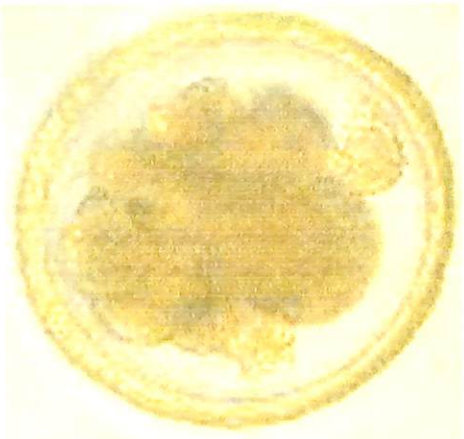


10. : Figure showing proces of inoculation of infection in a experimental rabbits.

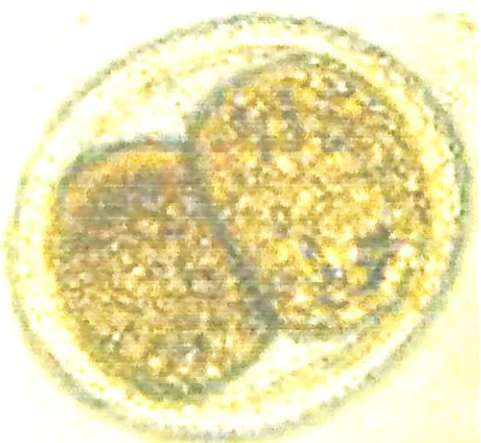
11. : Figures showing sequential development of *Toxocara canis* egg.



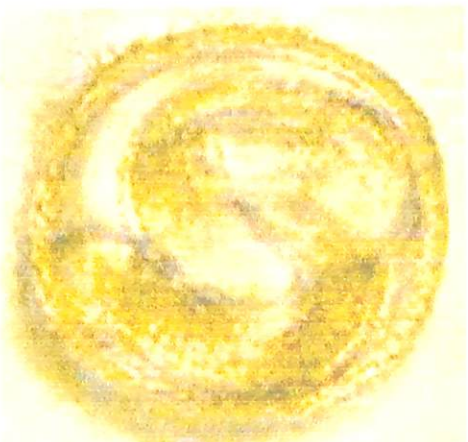
Egg in 4 cell stage of embryonic mass



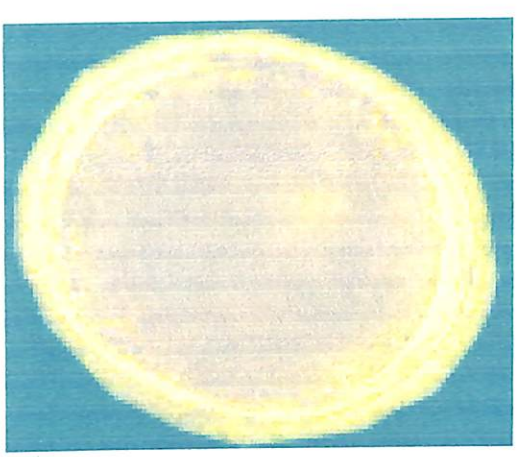
Multiple stage of embryonic mass



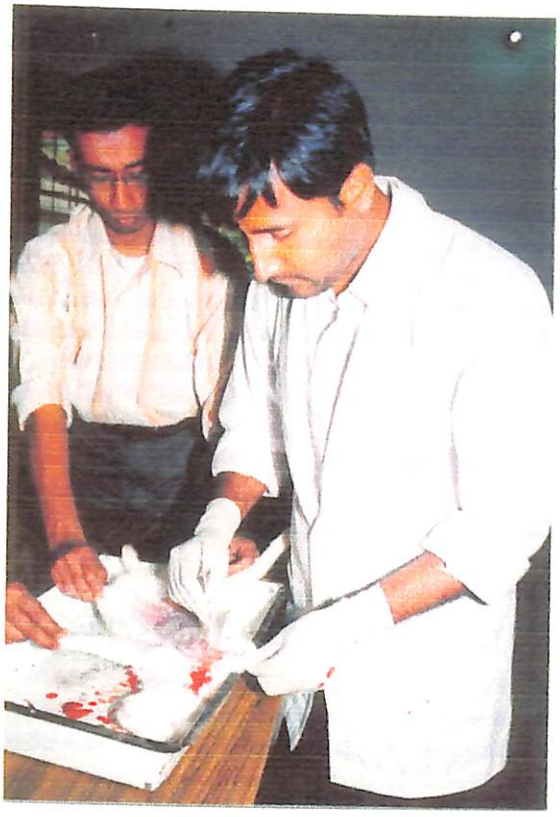
Egg in 2 cell stage of embryonic mass



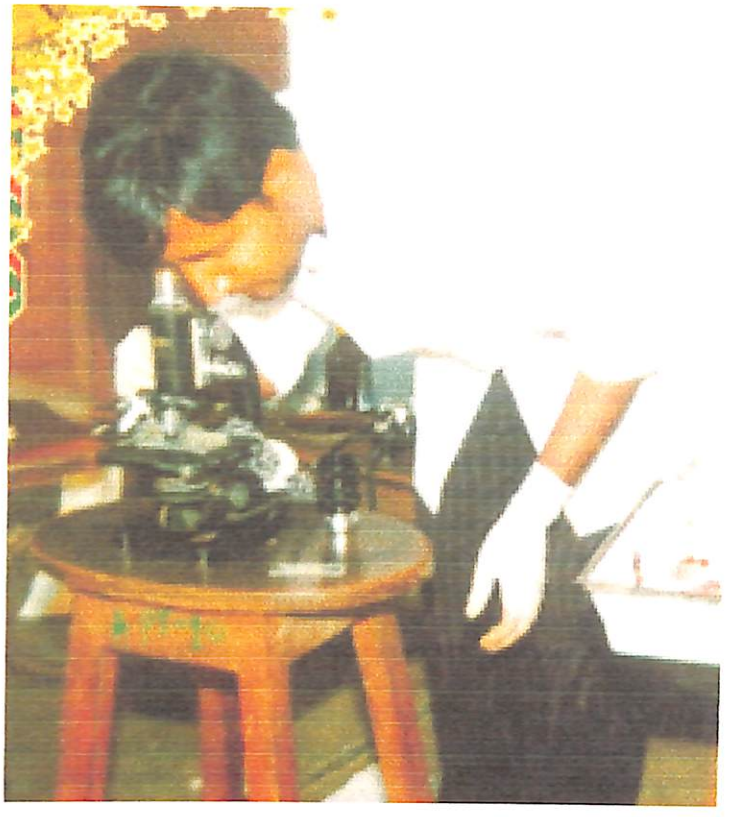
Egg with developed infective larvae (L_2)



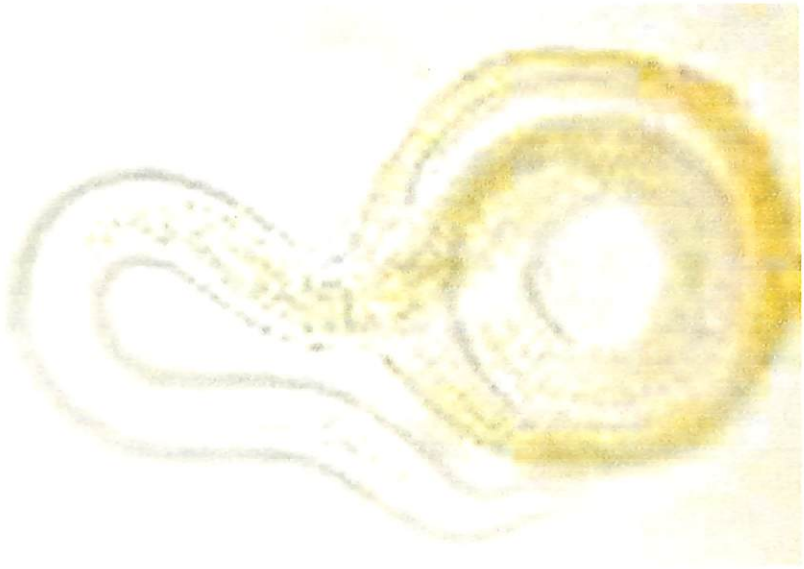
Unembryonated Egg



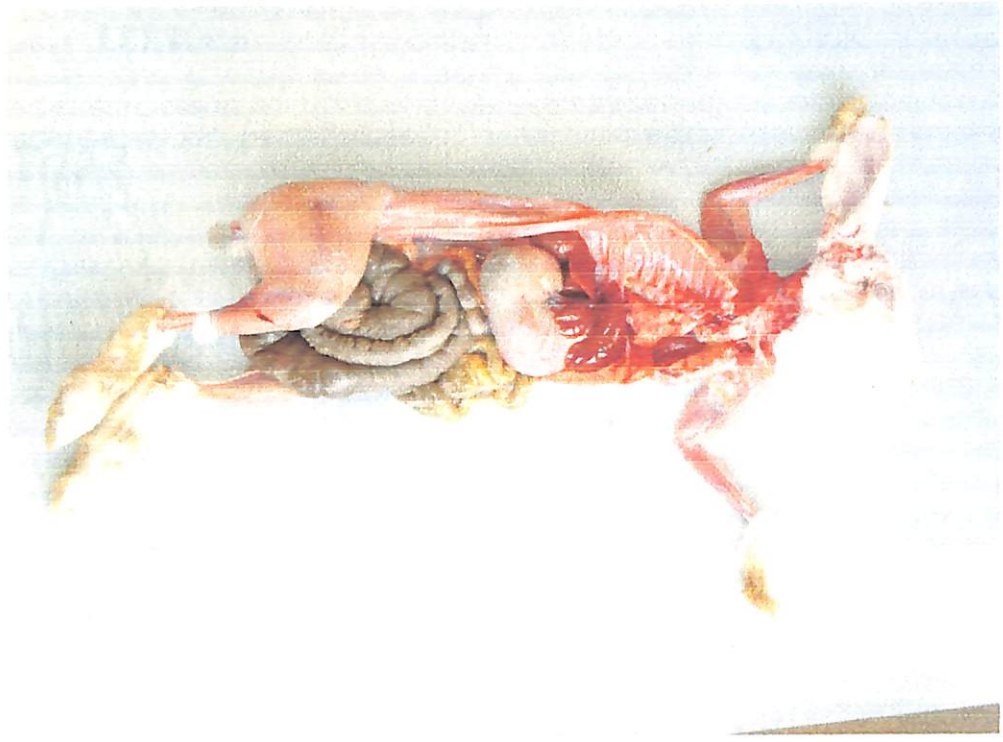
12. : Photograph showing disection of rabbit on day 7 post infection of *Toxocara vitulorum.*



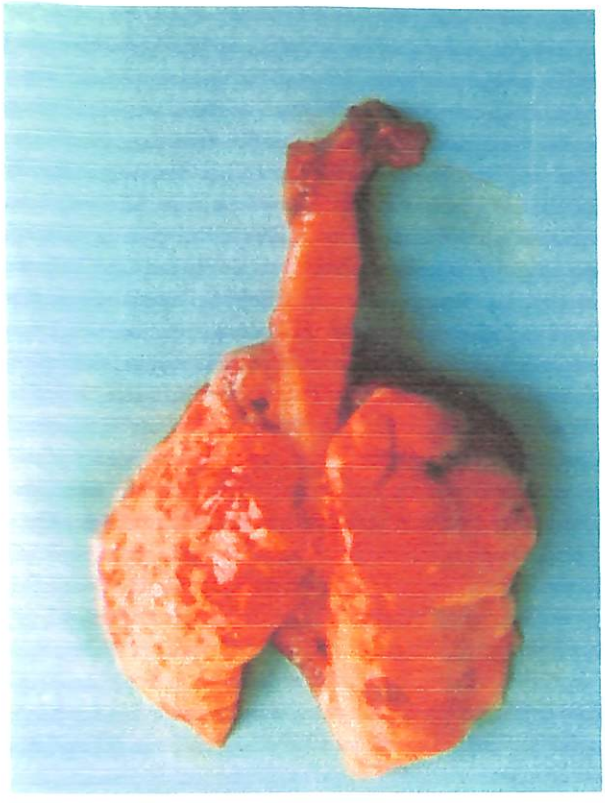
13. : Photograph showing Examination of intestinal content of rabbit.



14. : Figurs showing infective larva coming out of egg.

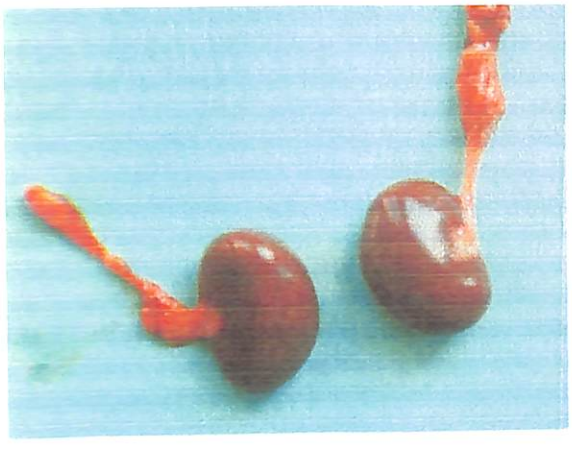


15. : Photograph showing internal organ of a rabbit of control group.

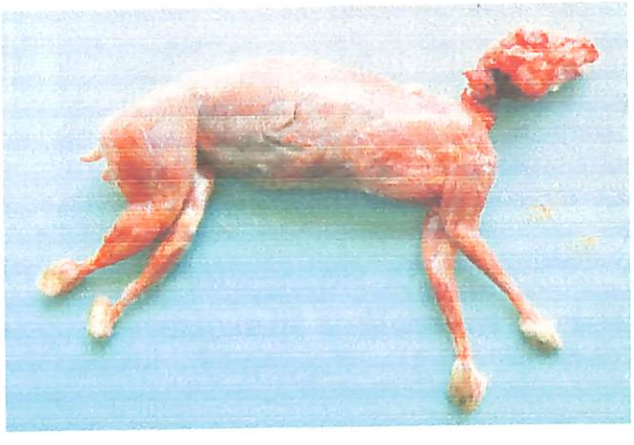


LUNGS

LIVER

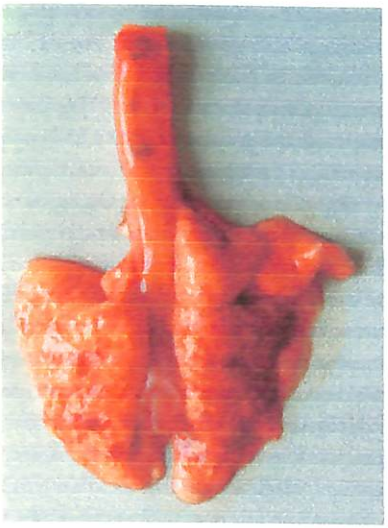


KIDNEY

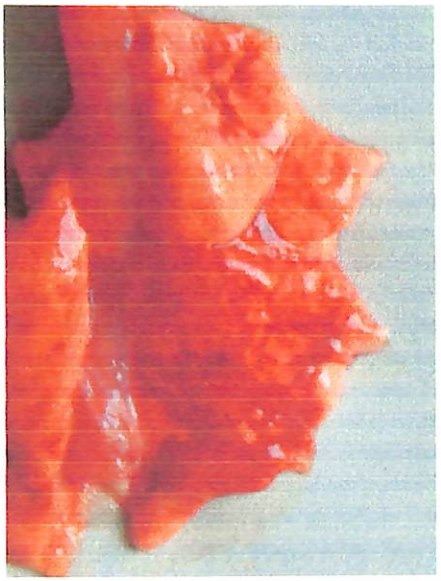


MUSCLES

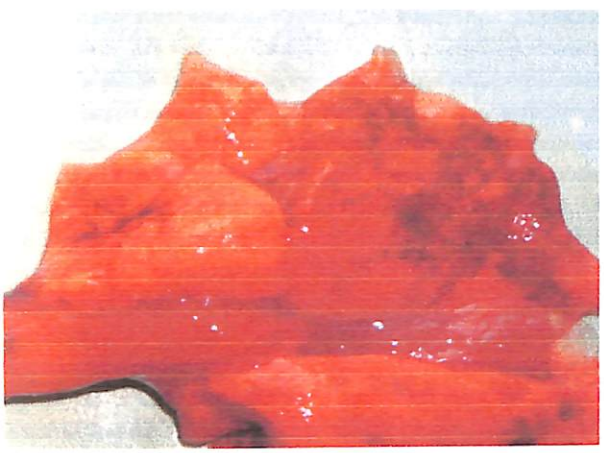
16. : Figures showing gross changes in various organs of infected rabbit on day 7th of post inoculation of Toxocara vitulorum infection.



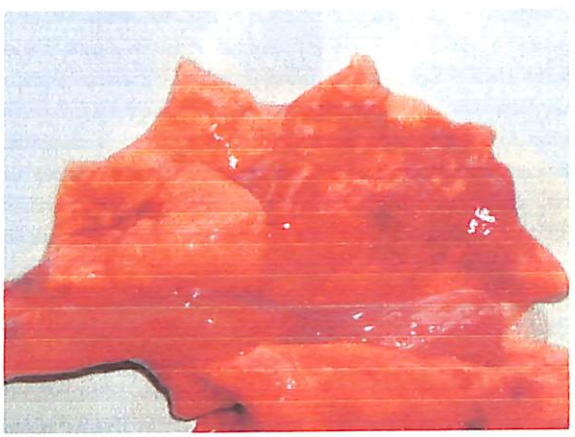
Photograph showing haemorrhagic Lesion (day 2 p.i.)



Photograph showing yellowish foci (day 2 p.i.)



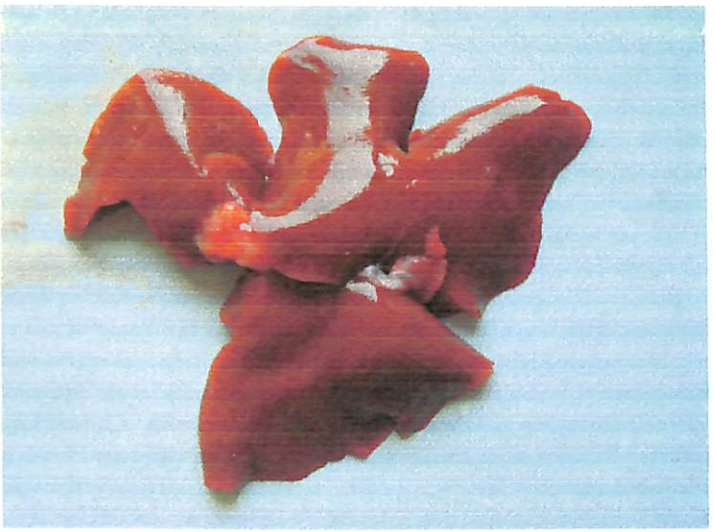
Photograph showing brownish haemorrhagic spots (day 7 p.i.)



Photograph showing prominant yellowish foci (day 7 p.i.)

17. : Photographs showing gross changes in lungs on 2 and 7 day of post infection of *Toxocara canis*.

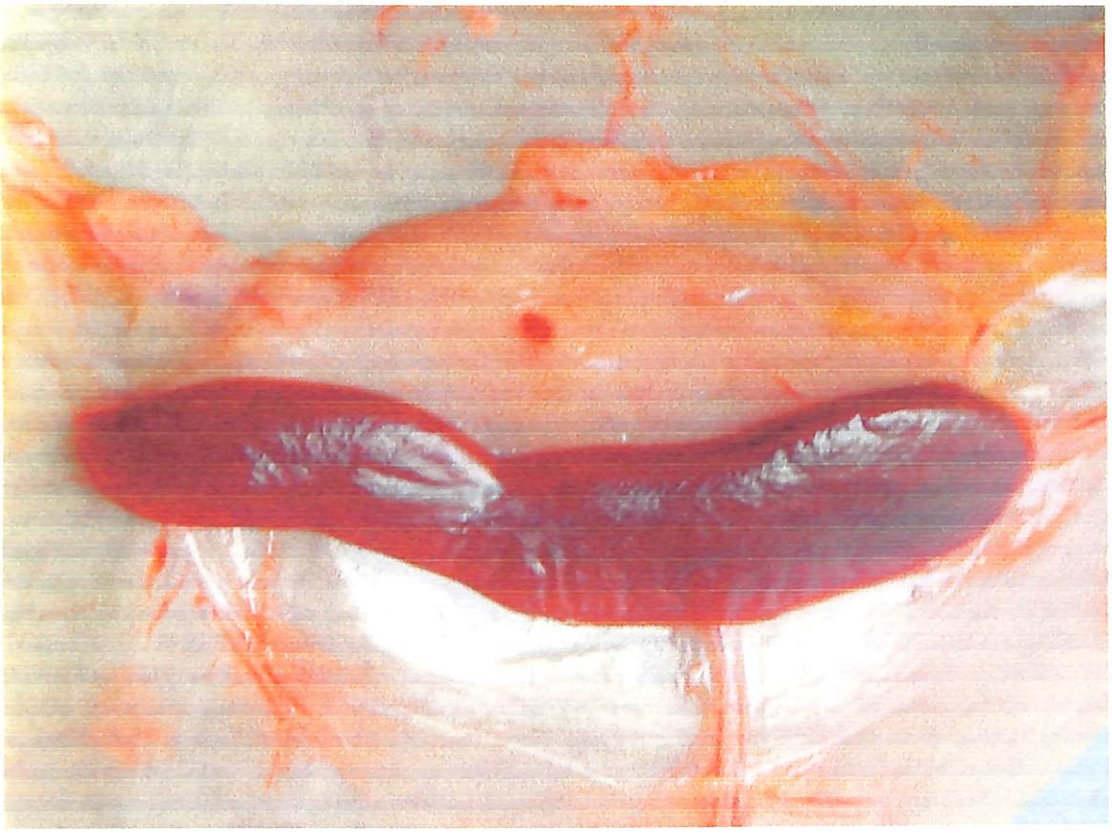
* p.i. = post infection



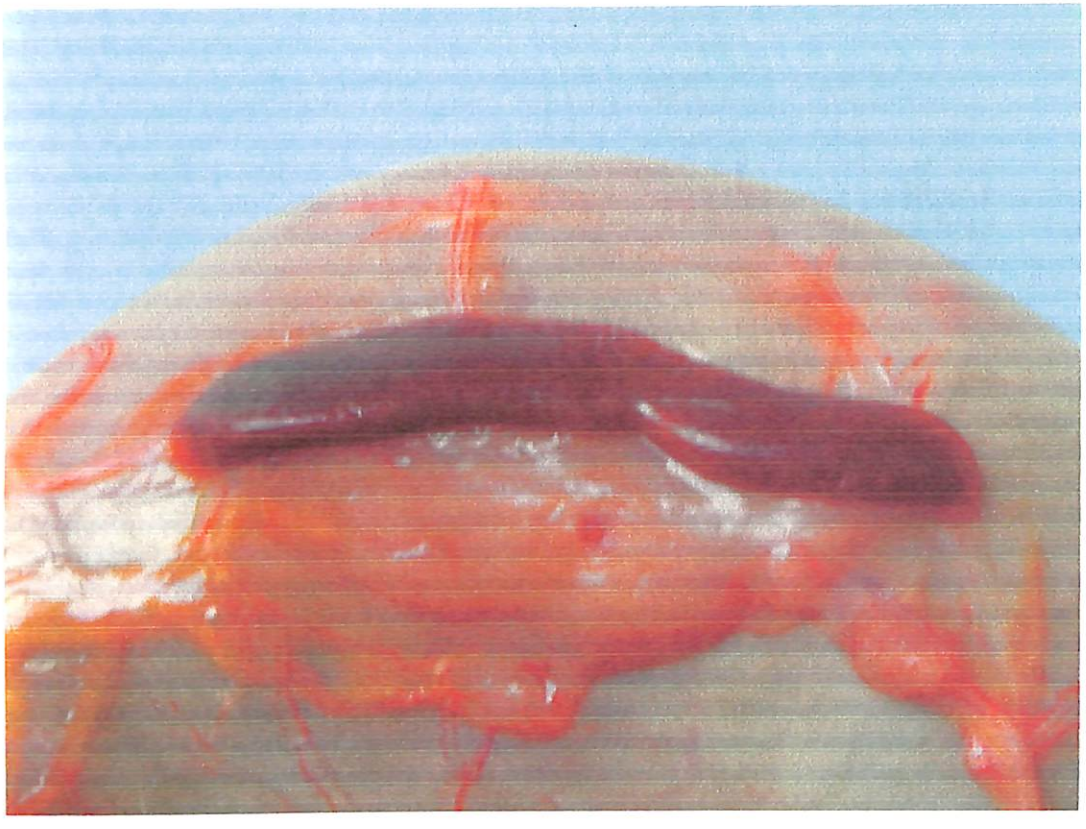
18. : Photograph showing liver infected with *Toxocara canis* on day 2 of post infection.

19. : Photograph showing Yellow and brownish hemorrhagic spots in liver infected with *Toxocara canis* on day 7 p.i.

20. : Photograph (in higher magnification) showing haemorrhagic spots in liver on day 7 p.i. of *Toxocara canis* infection.

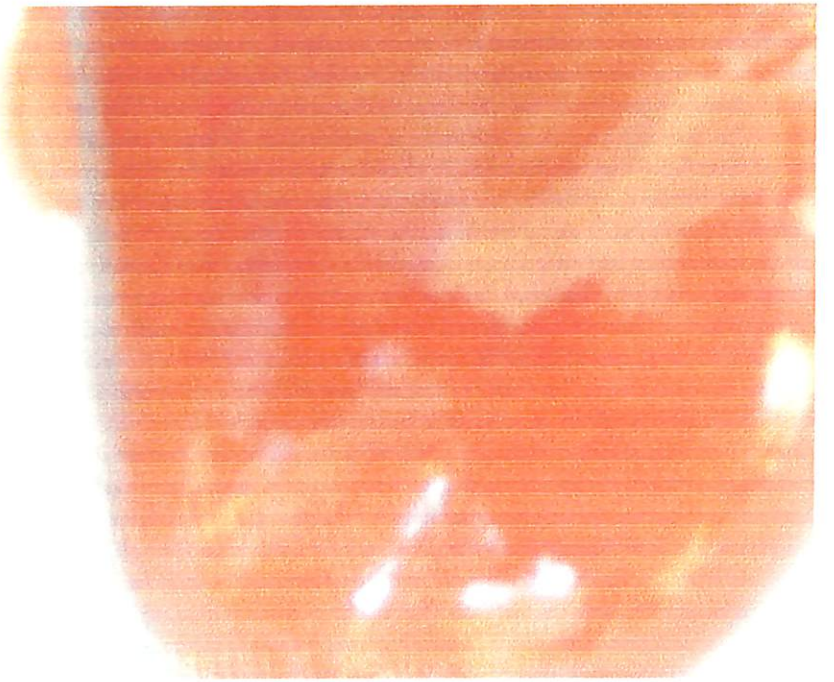


21. : Photograph showing almost normal appearance of spleen on day 2 of post infection of *Toxocara vitulorum*.

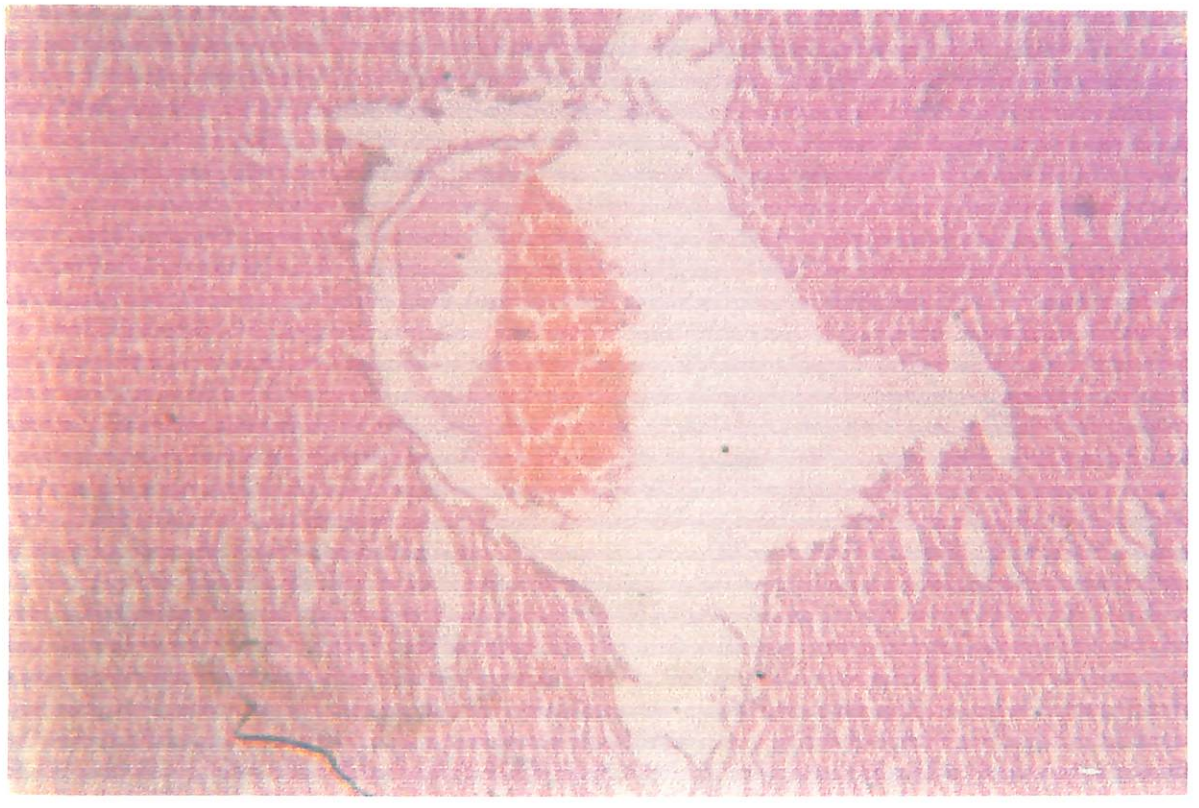


22. : Photograph showing no change in marphology of spleen on day 2 of post infection of *Toxocara canis*.

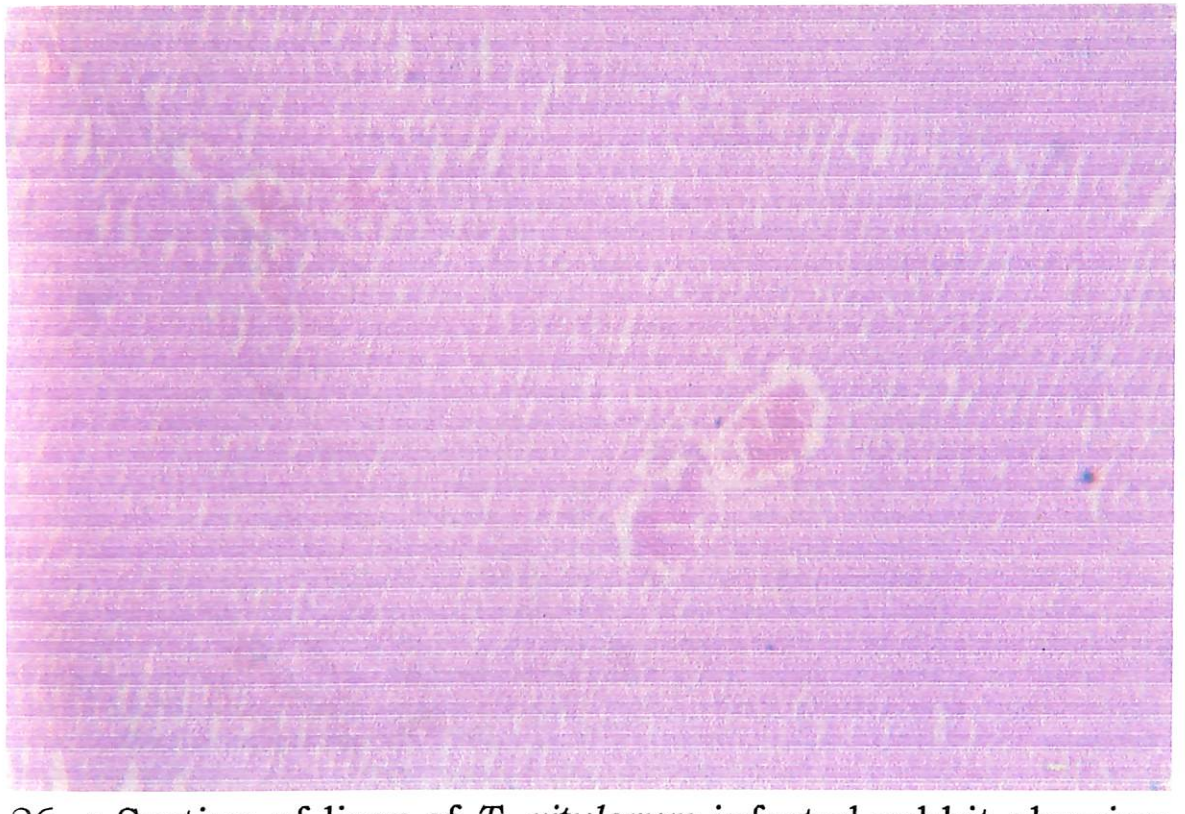
23. : Photograph showing enlarged kidney on day 7 post infection of *Toxocara canis*.



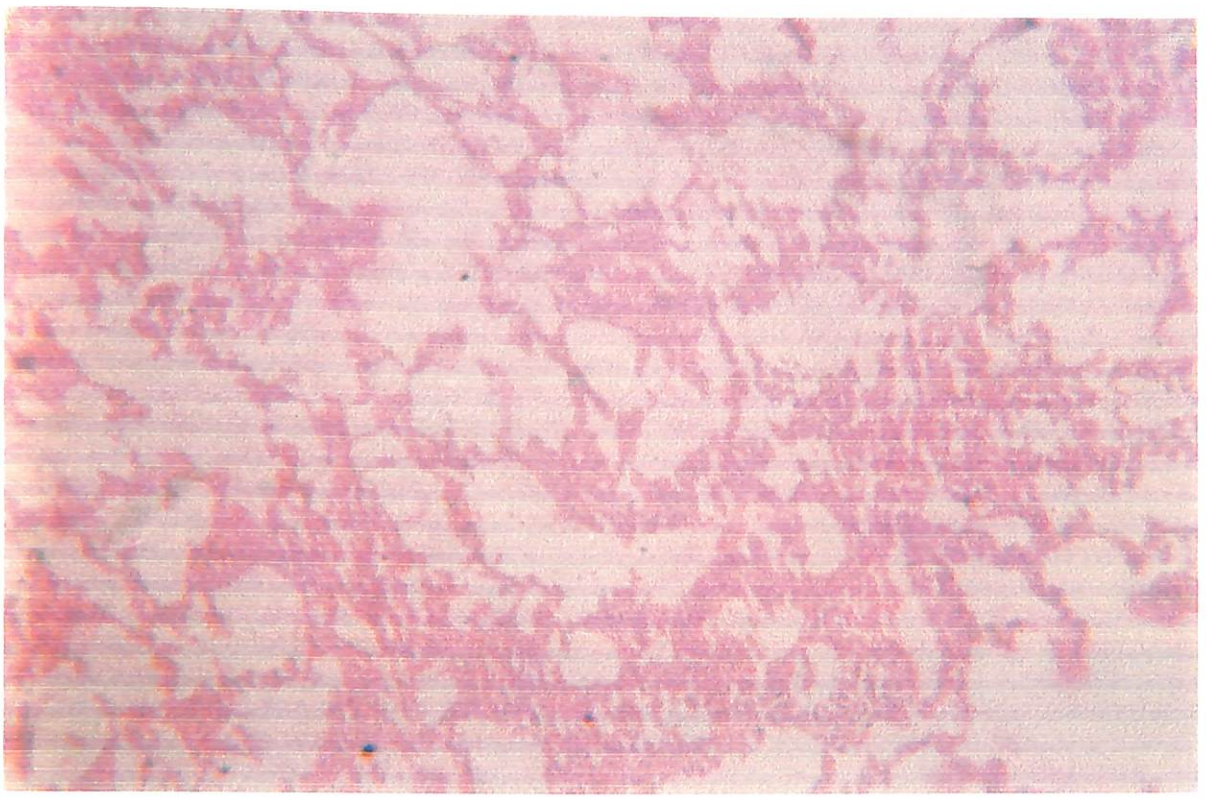
24. : Photograph showing haemorrhagic pale spots at higher magnification in kidney on day 7 post infection of *Toxocara canis*.



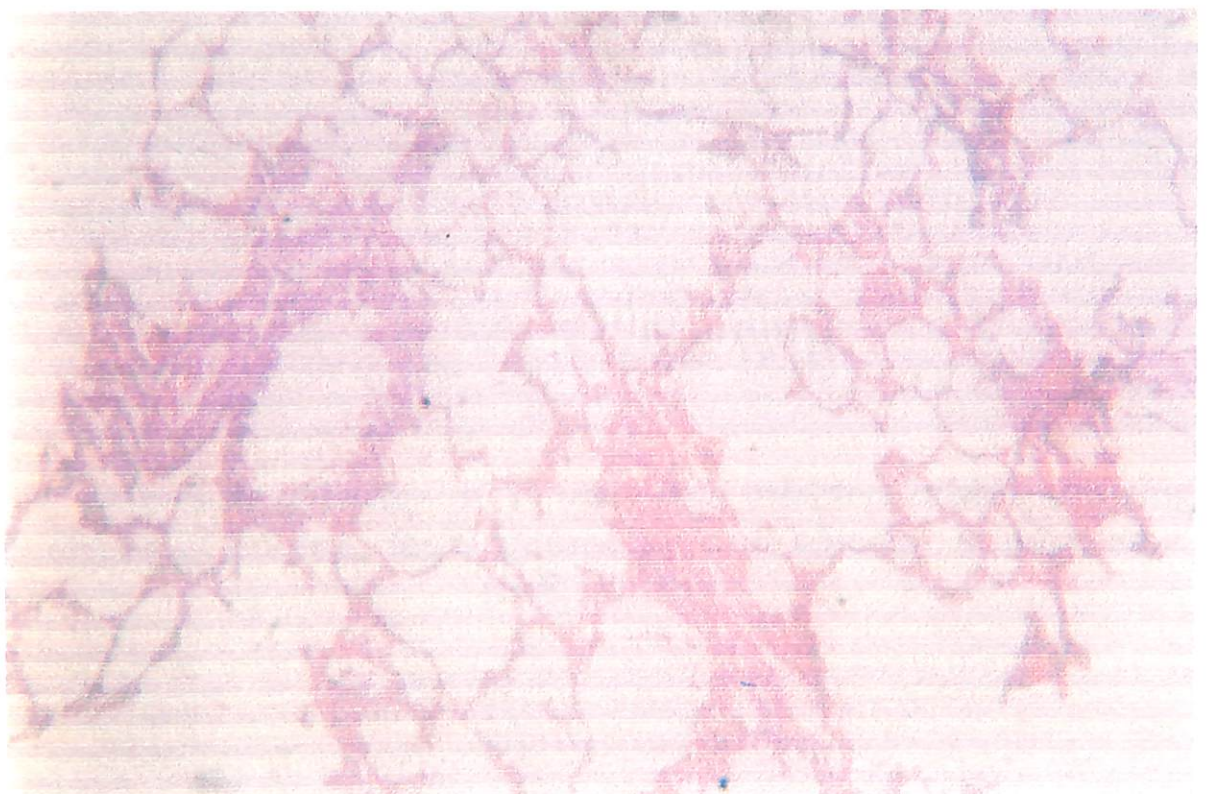
25. : Section of liver of *T. canis* infected rabbit showing dilatation of cental vein filled with haemorhagic contents and disorganised hepatic cords (H.E. X 100).



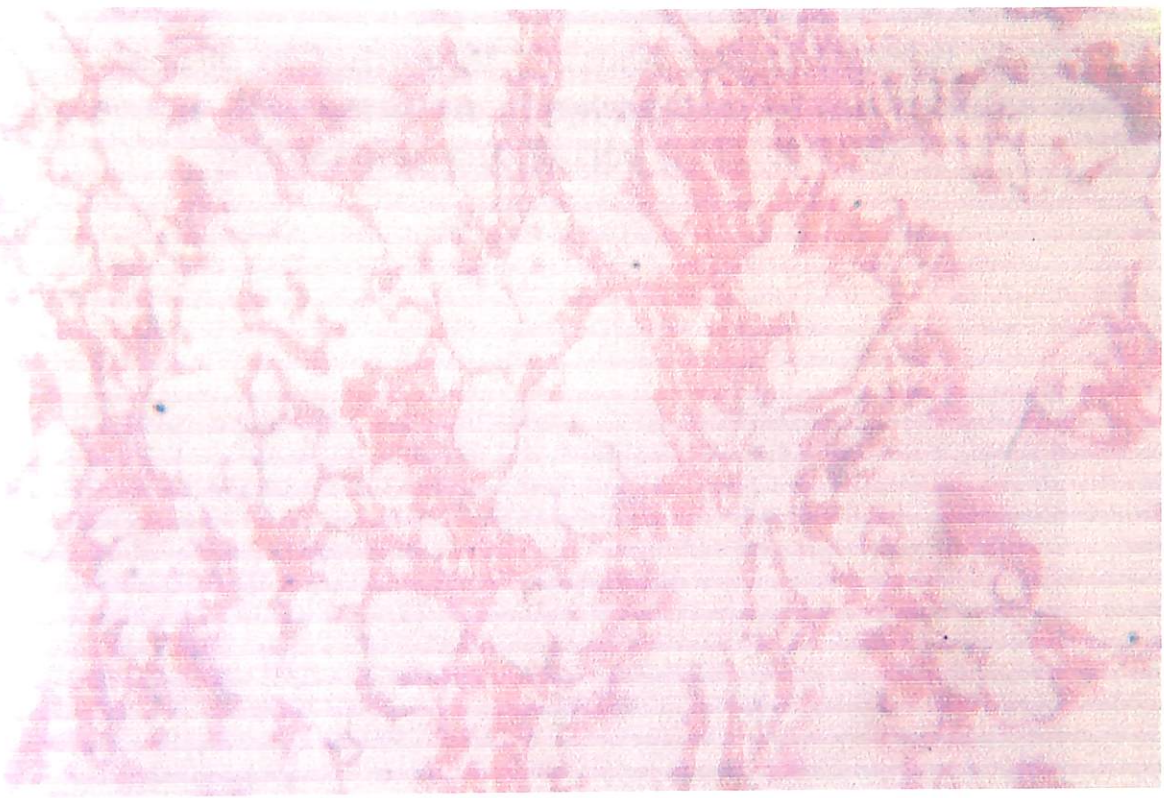
26. : Section of liver of *T. vitulorum* infected rabbit showing degenerative changes of hepatic cells with dilatation of central veins filled with blood cells (H.E. X 100).



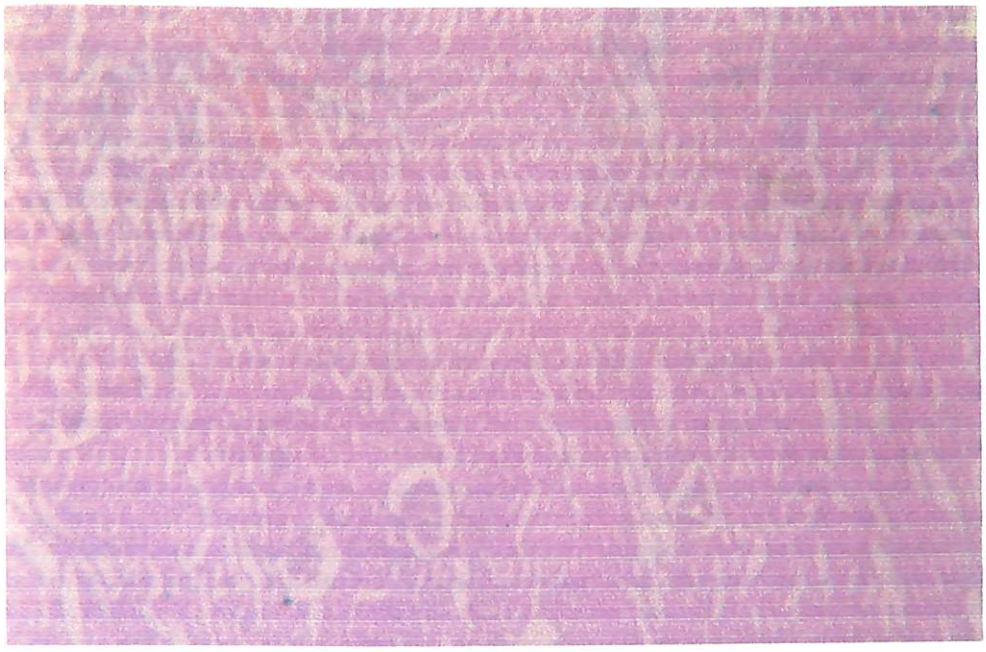
27. : Section of lungs of *T. canis* infected rabbit showing thickening of alveolar septa, bronchioles, and compensatory emphysema (H.E. X 100).



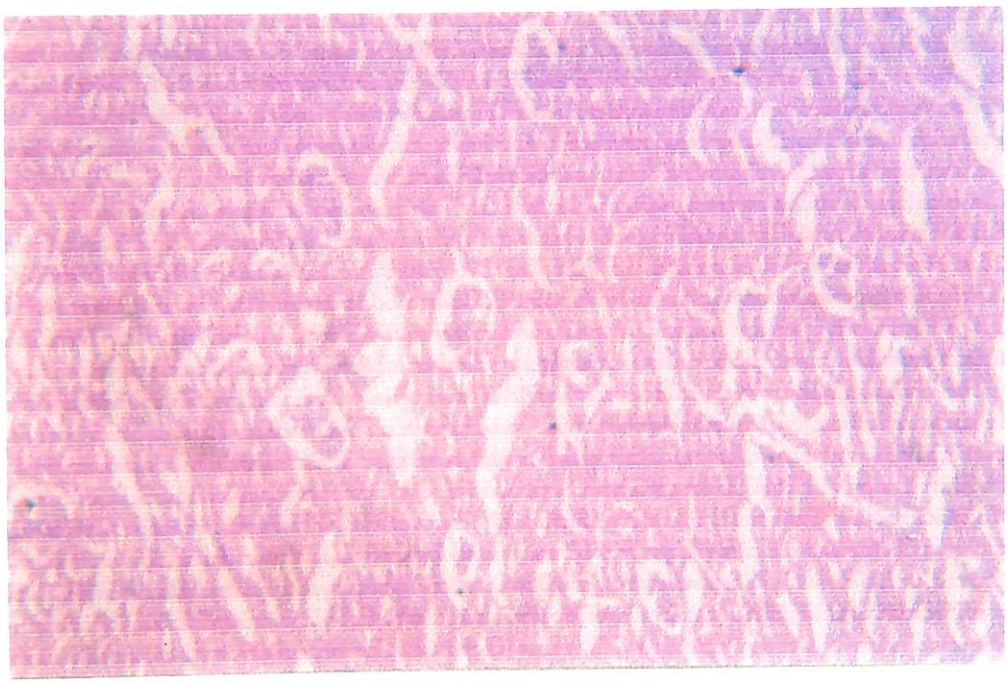
28. : Section of lungs of *T. vitulorum* infected rabbit showing a changes of bronchopneumonia and emphysema of alveolies (H.E. X 100).



29. : Section of lungs of *T. vitulorum* infected rabbit showing changes of edema, focal peumonia and alveolar emphysema. (H.E. X 100).



30. : Section of kidney of *T. canis* infected rabbit showing glomerular degeneration of epithelium of the tubules and glomerular capillaries (H.E. X 100).



31. : Section of kidney of *T. vitulorum* infected rabbit showing infiltration of inflammatory cells in the interlobular septa of cortical regions associated with degenerative changes in the lining epithelium of cortical tubules (H.E. X 100).

CHAPTER - V

DISCUSSION

DISCUSSION

Toxocariosis is an important disease of newborn calves and pups. Canine toxocariosis is one of the major zoonotic problems of considerable magnitude and important throughout the world on account of increasing trend of pet keeping, while higher prevalence rate of calf hood mortality shares the maximum significance of bovine toxocariosis in dairy industry. Although a variety of methods for controlling these diseases are under investigation in frame of very modern approaches but still the frequency of prevalence of these diseases are alarming. The present scenario required many more attempts on baseline studies regarding transmission of toxocarid infection in various paratenic hosts and the damages caused during migration of the development stages of these worms to evolve better and latest prophylactic or control measures. Considering the scanty reports available, the migratory behaviours of *Toxocara vitulorum* and *T. canis* larvae were studied along with gross and histopathological changes and larval recovery in various tissues of rabbits inoculated at the rate of 5000 egg/rabbit among 2 groups of animals respectively along with a control. Blood samples were collected to carryout haematological studies. The changes were observed on 2nd, 7th, 14th, 21st and 30th post infection in each group. Studies on incidence of toxocariosis in bovine calves and pups situated within locality of Patna and surrounding areas.

In present investigation, prevalence of *Toxocara vitulorum* and *Toxocara canis* was estimated to be 80.47 and 42.17% respectively in local bovine calves and pups population respectively of Patna on the basis of faecal examination (table1). Toxocariosis in pups and bovine calves are universal throughout the world, as various communications have been reported on the prevalence of toxocariosis from India and abroads. Anwar *et al.* (1996) investigated 63.8% toxocariosis in buffalo claves in Pakistan and Anene *et al.* (1996) observed 31.5% *Toxocaris canis* infection in Nigeria. Luty and Miagajska (1999) described that commonest parasite of dogs is *Toxocara canis* and 31.5% frequency of this infection in dog population of Germany. Martinez Barbadoza *et al.* (1998) recorded 21.2% prevalence of toxocariosis in pet dogs of Mexico City. Where as Overgaauw and Boersema (1998) reported that 21% of adult and 48% of pup population were infected with toxocariosis in the Netherlands. In India also Bharkhad *et al.* (1999) recorded 34.94% and 7.8% of T*oxocara vitulorum* infestation in faecal sample of cattle and buffalo cavles respectively in Marathwada. Grover *et al.* (2000) described that toxocariosis is soil transmitted disease as they found eggs of *T. vitulorum* in soil sample in and around Chattisgarh. Srinivasan Rao *et al.* (2000) recorded 40.09% of overall incidence of *T. vitulorum* in various age groups of buffalo calves. Rajkhowa and Hazarika (2001) recorded 37.09% incidence of *T. vitulorum* in female bovine calves of Assam. Katoch *et al.* (2002) evaluated 14%

prevalence of *T. canis* during coprological and faecal examination of pups' population of Himachal Pradesh. In our neighbouring country Bangladesh Islam *et al.* (2005) recorded 42% prevalence of toxocariosis in rural bovine population. Concerning to present result Pratibha *et al.* (2004) observed, incidence of *Toxocara vitulorum* in cow and buffalo cavles in Patna and its surrounding area was 38.33% and 41.60% respectively. Regarding *T. canis* infection, Sharma *et al.* (2005) recorded frequency of *T. canis* in 32.13 and 35.01% in pet and stray dog population of Patna respectively.

The variation on the rate of incidence depends upon different pattern of animal husbandry, age group of animals, geographical situation, climatic condition, nutritional status and schedule administration of anthelmentics and chemoprophylaxis. Hygiene and sanitation of the habitat area and pet keeping plays a vital role in decreasing the frequency of infection rate. In present study higher prevalence rate was recorded both in case of bovine calves and canine pups, might be related that faecal samples were collected only from a certain age group of (20-45 day) calves and pups up to 3 months when lactogenic transmission of ascariosis is more evident along with soil transmission.

Further this study was conducted to evaluate the effect of sex on *Toxocara canis* and *Toxocara vitulorum* (adult worms) recovered from intestines 5 hosts. However there was no significant ratio noted between recovered male or female

worms both in case of canine or bovine toxocarid worm but male were found more in numbers in comparison to females.

Studies on migratory behaviour of toxocarid larvae :

Migratory larvae of *Toxocara* in various organs of host, generate considerable vascular and cellular responses in invaded areas. It attempts to destroy the tissue by formation of migratory tract and then endeavour the processing or cell division and infiltration, to repair the damage created in affected areas. The degree of tissue damage, irritation and tissue sensitivity determines the course of termination of vascular responses in host body. The migratory larvae also affect the humoral responses of the body.

Various haematological changes during migration of *T. vitulorum* and *T. canis* larvae in rabbits :

The present study describes various haematological changes in rabbit experimentally infected with 5000 embryonated eggs of *Toxocara vitulorum* on day 2nd, 7th, 14th, 21st and 30th day of post infection (Table 4 and 5).

During study of haemoglobin (gram %) in rabbits infected with *T. vitulorum* falled down continuously and significantly up to 21st day of post infection However, improvement in Hb% was observed on 30th day post infection, present findings are also in close agreement with the reports of Lau and Singh (1985) Panday and Mishra (1985) and Usharani Devi *et al.* (2000) as they also observed reduction in Hb% in cows and buffaloes associated with natural infection of *T. vitulorum* however Neves

t al. (2004) recorded no significant changes in buffaloes nfected with *T. canis*.

Significant leucocytosis was marked on 2nd day p.i. of *T. itulorum* in rabbits and was recorded up to 21st day of post nfection. After 21 day p.i. slight resolution in leucocytosis was bserved on 30th day post infection.

Homologus to present results Elabdin *et al.* (1975) also ecorded significant increase in various leucocytes in calves nfected with *Neoascaris vitulorum*. Lau and Singh (1985) also eported leucocytosis in buffalo calves naturally infected with oxocariosis. Lukes (1985) also observed marked increase in eucocytes during experimental larval toxocariosis in rabbits, which closely resembles with the findings of present study. Thakur *et al.* (1998) also suggested that under lab condition experimental inoculation ascarid ova show gradual development of leucocytosis.

Gradual decrease was also observed in the values of pack cell volume (PCV) up to day 21 of post infection then slight recovery observed on day 30 p.i. confimatory to present finding Lau and Singh (1985) Pandy and Mishra (1985) and Usharani Devi *et al.* (2000) also reported significant fall in PCV values during natural toxocariosis in calves.

The haematological changes in rabbits following experimental infection revealed significant fall in values of lymphocytes and neutrophils. El-Abdin *et al.* (1975) also observed lymphopenia in calves infected with *Neoascaris*

vitulorum which is similar to present finding but neutrophilia was also evident in his studies which is dissimilar to present result. Lau and Singh (1985) reported lymphocytosis while neutrophilia was observed by Luke (1985) during natural and experimental infection of toxocariosis in bovine calves, which is not similar to present result. Identical to present investigation Sinha *et al.* (1987) reported combined decline in values of neutrophil and lymphocytes up to 7 and 11th days of post infection respectively during experimental infection of *T. vitulorum* in mice.

From the results it was evident that circulating eosinophilia raised significantly on day 2 following infection in rabbits with *T. vitulorum* and reached maximum (27.40%) on day 14th. Eosinophilia is characteristic feature of any ascarid infection and Panebianeo (1954) observed eosinophilia up to 30% in calves after infection of *T. vitulorum.* Eosinophilia is evident feature in many parasitic infections, as eosinophils become prominent constituent of the cellular exudates in various infection of respiratory system. El-Abdin *et al.* (1975) marked eosinophilia during toxocariosis in calves. Butterworth (1977) also demonstrated that eosinophilia plays significant role in immune system during helminthic infection.

Identical to present finding Lau and Singh (1985) Lukes (1985), Panday and Mishra (1985), Sinha *et al.* (1987) and Usharani Devi *et al.* (2000) also recorded maximum post infection eosinophilia (up to 22%) under natural or

experimental toxocariosis in calves or paratenic hosts respectively.

Results on the basis of blood picture of rabbits experimentally infected with *T. vitulorum* revealed non-significant changes in the values of monocytes throughout the study. Lau and Singh (1985) and Sinha *et al.* (1987) also recorded non-significant alteration in the counts of monocytes between pre and post infection observations.

Summarizing the haematological changes in rabbits following experimental infection of *T. vitulorum* revealed marked haemoglobinemia with reduction in PCV. Leucocytosis was accompanied with lymphopaenia, neutropaenia and significant eosinophilia was also marked up to 21 days of post infection. On day 30th observation there was resolution of infection and recovery was noticed in almost blood parameters. This might be suggested that histotropic migration of infective larvae of *T. vitulorum* is responsible for many changes in immune or humoral responses in blood vascular system. The degeneration of cells and allergic responses is also being manifested during migration of larvae.

Analysed data pertaining to various haematological observations on administration of 5000 eggs of *Toxocara canis* in experimental rabbit at 2nd, 7th, 14th, 21st and 30th days of post infection revealed significant fall in the values of Hb% from control groups up to day 14 p.i. Thereafter considerable

:covery observed on day 21 and 30 p.i., however these values ere also lesser than healthy (control) animals.

Vossman and stoye (1986) reported that all massively ıfected puppies with experimental *T. canis* died within 22-49 ays of age and were found subfreezing with severe anaemia. ao and Suryanarayana (1995) also revealed marked decrease ı haemoglobin and erythrocytic count level in affected dogs ith natural toxocariosis. Identical to present result Tudor 2004a) also demonstrated decrease in RBC in the initial state f experimental infection with embryonated egg of *T. canis* in abbits.

Blood picture of experimental rabbits further revealed ıarked increase in the values of total leucocytic count up to ay 14 of p.i. and slight recovery was observed on day 21 and 0 of p.i. Present result corroborates with the reports of rokopic and Figallova (1982b) and Lukes (1985). Sugane and)shima (1985) also found increased and peak of T.L.C up to 4 day of p.i. significant leucocytosis was also observed by /ossmenn and Stoye (1986). Thakur *et al.* 1988 observed radual development of leucotytosis under lab condition of xperimental inoculation of ascarid ova. Analogous to present inding Tudor (2004a) demonstrated that embryonated eggs of *T. canis* produced, decrease in TLC in 1st hrs afterwards significant increase followed up to day 20 p.i.

Blood pictures of rabbits experimentally infected with *T. canis* showed significant decrease in PCV values throughout

experimental period than control group. Rao and yanarayana (1995) indicated marked decrease in PCV value ing natural toxocariosis in dogs.

Marked lymphopaenia, neutropaenia, eosinophila was erved during haematological examination of rabbit infected h 5000 embryonated eggs of *T. Canis* in present result. yden and Van Kruiningen (1975), Butterworth (1977), kopic and Figallova (1982b), Sugane and Oshima (1982), kopic and Figallova (1983), Jenkins and Richard (1984), re also observed rise in eosinophilia from day 7th to 21 in perimentally infected mice with *Toxocara canis* eggs. They rther indicated that eosinophilia closely related to cell-ediate immune mechanism in *T. canis* infected mice, and ay vital role in producing immunity towards infection. Sugan d Oshima (1984), further reported that the increasing mber of circulating eosinophilia after infection did not show lationship with total number of larvae recovered in *T. canis* fected mice. Lukes (1985) also noted leucocytosis, sinophilia and neutrophilia in rabbit infected with 5000 mbryonated egg of *T. canis* which partially supporting the resent result, while Sugane and Oshima (1985) noted that eutrophil did not increase significantly in mice inoculated ith *T. canis*. All the above authors found increase value of sinophilia up to 14th day of post infection. Vossmenn and toye (1986) observed most obvious change in blood picture as eosinophilia in puppies infected with *Toxocara canis*.

Person *et al.* (1989) also demonsrated haematological responses in cats experimentally infected with *T. canis* larvae and recorded higher circulating eosinophil level in blood. Rao and Suryanarayana (1995) and Thakur *et al.* (1998) noted serious eosinophilia during natural and experimental toxocariosis respectively. Tudor (2004a) reported high level of comparative fluctuation in eosinophilic counting which found started increased from 6 hrs. p.i. and highest level reach at 21 day p.i. in guinea pigs during experimental infection of *T. canis* Revajova *et al.* (2006), however evaluated immunosensitivity in lambs towards multiple toxocara infection and observed that leucocytes and neutrophills were not significantly higher 12 hr. p.i. however greater no. of lymphocytes were observed in peripheral blood. Confirmatory report to present finding reported by Samanta (1989) as he demonstrated lymphopaenia and neutropaenia and eosinophilia in albino mice experimentally infected with *T. canis* larvae.

The degree of changes in monocyte count remained non-significant throughout the experimental period, identical to this finding Samanta (1989) also unable to found any variation in monocyte values.

Studies on larval counting in different organs during infection of *T. canis* and *T. vitulorum* :

Table -8 and 9 in present study described the counting of migratory larvae in different organs of experimental rabbits following oral infection of 5000 embryonated egg of *T. vitulorum*

and *T. canis* respectively. The larval count of *T. vitulorum* was observed maximum in liver followed by lungs, intestinal content and skeletal muscles however no larval recovery was noted from tissue of kidney and spleen. In case of liver, larvae of *T. vitulorum* was found maximum on day 2 p.i. thereafter a gradual decrease was noted. In lungs and intestinal content, highest larval count was observed on day 7th post infection then followed decrease in number of larval count up to the end of experiment. While larvae appeared in skeletal muscles on concluding day i.e (30th) of observation. Chauhan *et al.* (1974) noted highest recovery of larvae of *Neoascaris vitulorum* in liver of albino rates and chicken, Paul *et al.* (1981) however found considerable number of *T. vitulorum* larvae in uterus of pregnant mice. Pramanik *et al.* (1994) studied the migratory behaviour of *T. vitulorum* larvae in rabbit and observed continuous higher larval count in liver from day 3 to 42 p.i. (highest on day 3 p.i.) followed by in lungs from 3 to 63 day p.i. (highest recovery on day 7 p.i.) and in muscles from 14 to 63 days p.i. (highest recovery on day 28 p.i.) however no larvae were recovered from intestine, spleen and brain. This is almost identical to present result. Moyo (2002) also observed maximum numbe of migratory larvae were recovered from liver from day 1 to 10 p.i. where as in lungs day 3 p.i Paula *et al.* (2005) eliminated highest number of larvae (*T. vitulorum*) in mice from liver and lungs at 7 hrs p.i. and in large intestine on day 4 after the challenge of larvae where as in muscles it

recovered only on day 30th p.i. It was suggested that the migratory behavior of *T. vitulorum* larvae and their persistence in various tissues might related with possibility of visceral larval migran in rabbit.

Table -9 reveals number of larvae recorded from different body organs of rabbit infected with *T. canis*. The study indicated that the maximum number of larvae recovered from liver on day 2nd p.i. followed by lungs on day 7th p.i. during examination of intestinal content presence of larvae observed only on day 7th and 14th p.i. and maximum number of larval count was noted on day 7th p.i. During examination of skeletal muscles maximum number of larval count observed on day 30 p.i. however presence of larvae also noted on day 21 p.i. Analysing the present finding Prokopic and Kilabanova (1988) found distribution of migratory larvae of *T. canis* in liver (82%), lungs (80%) and brain (63%) and was maximum in liver, lungs, and leg muscles on day 2, 4, 28 p.i. respectively in experimentally infected white mice with *T. canis*. Peppersack (1981) and Min (1982) found the proportion of larvae of *T. canis* gradually decrease in muscles of mice after 5 day p.i. which differs to present observation. Prokopic and Figallova (1982) also observed numerous migratory larvae of *T. canis* in liver, lungs and muscles in experimentally infected mice. Results are also supported by the report of Sugane and Oshima (1983) who found total large number of larvae in liver after re-infection of larvae (L_2) in mice. Sharma and Bhatia

(1983) also observed that higher larval count in the liver form 12 to 24 hrs onwards inoculation and mainly in intestine after 24 hrs. p.i. in mice experimentally infected with *T. canis* larvae. Hyat and Hyat (1984) studied migration of ascarid larvae in lambs and complete migration (3^{rd} and 4^{th} stage larvae) were recorded from lungs and intestine.

Confirmatory to present observations Abo-shehada *et al.* (1984-85) also observed that L_2 larvae reached in various parts of stomach and large intestine within 2 hr. of p.i. the posterior halves of intestine was the preferred site of *T. canis* infection in mice. Lohmann (1985) also reported that the larvae of *T. canis* mainly detected in muscles and brain of mice at 20 to 160 day p.i. The number of muscle larvae decreased more rapidly than infected brain Gargili *et al.* (1999) admninstered 5000 embryonated *T. canis* egg orally and found migratory larvae in liver, brain and lungs. Saeed *et al.* 2005 observed effective larval migration from lung to other tissue in arctic fox up to 150 day p.i. of *T. canis.* Identical finding to present result was also reported by Tudor (2004) as he also suggested that larval migration of *T. canis* was histotropic in rabbits and larvae disperse throughout the body and entered the myotropic and neutrotropic phase by the 7^{th} day of infection. The number of recoverable larvae declined gradually with periods of stable population.

Gross and histological changes during migration of *T. vitulorum* larvae in various organs of rabbits :

Tissue damages during migration of larvae of *Toxocara vitulorum* in experimentally infected rabbits with 5000 embryonated eggs were studied in various organs on day 2, 7, 14, 21 and 30th of post infection (p.i.), Gross changes in liver revealed enlargement in size of liver, white to pale coloured spots along with lodged larvae on the surfaces of liver up to day 21 p.i. Microscopic changes were those of degenerative types accompanied with structural changes in hepatic cells up to 21 days. Extensive haemorrhages and necrotic changes accompanied with infiltration of eosinophils and macrophages were recorded. Chauhan and Bhatia (1973) reported similar gross and histological changes in liver of poultry experimentally infected with *T. vitulorum*. Whereas Sinha *et al.* (1981) and Srivastava *et al.* (1988) observed similar changes in liver during experimental infection of *T. vitulorum* in mice. Pramanic *et al.* (1994) also noticed same gross and histological changes in liver during experimental infection of *T. vitulorum* in rabbits.

Grossly lungs showed petechial haemorrhages and pale foci on surface along with congestion and pneumonic changes. Microscopically, cut sections of lungs showed the engorged blood vessels and haemorrhagic migratory routes, larval sections and changes of emphysema in alveolar regions. Alveoli

were disorganized and bronchioles were dilated and changes of pneumonia were evident up to day 21 p.i. All the gross and histological changes in lungs are corborated with description given by Chauhan and Bhatia (1973) in poultry experimentally infected with *T. vitulorum*. Whereas Sinha *et al.* (1981) and Srivastava *et al.* (1988) observed similar changes in lungs in mice experimentally infected with *T. vitulorum*. Pramanic *et al.* (1994) also found emphysema and pneumonia in lungs of rabbits during experimental infection of *T. vitulorum*.

In present study, structural changes were not noticed in kidneys except slight enlargement in size up to day 14 p.i. while on day 21 and 30 petechial haemorrhagic spots were seen on the surfaces of kidney. Histopathologically blood vessels in cortex and medulla were dilated. Congestion in blood vessels was also prominant due to accumulation of erythroytes. Cells of malpighian tubules were hypertrophied. Identical findings were also reported by Srivastava *et al.* (1988) in kidney of mice and Pramanic *et al.* (1994) in kideny of rabbits both experimentally infected with *T. vitulorum*.

Spleen was mildly enlarged in size along with changes of congestion and areas of haemorrhages were visualized during histological examination of spleen. Identical to present result Srivastava *et al.* (1988) also noted mild congestion and haemorrhagic spots in spleen of mice infected with *T. vitulorum*.

Haemorrhagic spots were observed during gross examination of intestine up to 21 day p.i. but blood mixed faecal materials was observed in lumen of large intestine while microscopically desquamation of mucous membrane and lack of differentiation of parietal cells, hyperplasia and inflammation in submucosal areas with infiltration of mononuclear cells was noticed up to day 21 p.i. Chauhan and Bhatia (1973) noticed similar changes in caecum of poultry experimentally infected with *T. vitulorum.*

In skeletal muscles migratory tracts of the larvae observed on day 7 post infection surrounded by macrophages and eosinophils. Observation made by Prmanic *et al.* (1994) closely resembles to the organ and tissue level changes in rabbits striated muscles with present result.

Gross and Histological changes in various organs of rabbit due to migration of *T. Canis* larvae on various days of post infection :

The gross observation of liver in infected rabbit was noticed as enlargement in size, yellow spots and superficial attachment of numerous larvae up to 21 days of p.i. Histological examination revealed conspicuous migratory routes with infiltration of haemorrhagic content, neutrophils and eosinophils. There were many degenerative changes along with hepatostatosis were seen. Disorganisation of hepatic cell was also evident. Similarly Sharma and Bhatia (1983) also found degenerative changes due to presence of large number of

larvae in liver of chicken and albino mice within 12 and 24 hrs respectively infected with *T. canis*. Hyat and Hyat (1984) also reported pronounced damages caused by *T. canis* larvae in liver of lambs. Histologically Abo-Shehada and Herbert (1984-1985) observed that the larvae migrate rapidly from central veins to liver prenchyma. Identical to present study, Mondal (2002) demonstrated the numerous greyish milk spots on surfaces of liver and inflammatory foci in liver sections of rabbits infected with 5000 embryonated egg of *T. canis*. Tudor (2004) represented microscopical modification in liver, by leucocytosis, eosinophil and lymphocyte within blood vessels along with fatty degeneration and hepatostatosis. He also observed necrosis, massive cell destruction in cut section of infected liver. All these closely resemble to present results.

Grossly, congestion was observed in lungs of infected rabbits, various yelowish foci along with haemorrhagic lesions, emphysema, atelectasis and disorganised lungs architecture was found due to heavy larval burden while microscopically inflammatory reaction was noticed with infiltration of neutrophils, eosinophils and lymphocytes observed up to 21 day p.i. Pulmonary oedemas, emphysema, broncho pneumonia, congestion in alveoler capillaries were also marked during experimental studies.

Analogous to present result Abo-Shehada and Herbert (1984-85) observed that infective larvae of *T. canis* reached lungs tissues within 3 days and cause verminous pneumonia

with peticheal haemorrhages and congestion of alveoli. Mondal *et al.* (2002) also reported that the lung tissues of rabbit infected with *T. canis* larvae showed congestion, oedema, emphysema and infiltration of neutrophil, eosinophil, macropheges and giant cell. While Tudor (2003-04) also demonstrated the lesional image of lungs represented by acute haemorrhge in sub-pleural region, vicariant emphysema, haemorrhage on lung surface, atelectasis areas, however microscopically inflammatory process in alveolar region was recorded, which was surrounded by eosinophils and macrophages infiltration, which is characteristic of interstitial bronchopneumonia. Pulmonary edema and compensatory emphysema was also observed in rabbit infected with *T. canis* in present study.

Slight congestion was observed in the surface of kidneys and pale apperance on day 7th and 14th p.i. Microscopically lymphocytic infiltration in cortex and granular degeneration of glomerular capillaries was observed up to 14th days. Abo-Shehada and Herbert (1984-85) seen histologically active migration of larvae in kidney cortical region. Mondal *et al.* (2002) also found slight enlargement and pale appearance of kidney tissue of rabbit infected with *T. canis*. Tudor (2004) demonstrated that the congestive lesions on surface and yellow brownish area similar to present result. Evidence of lymphocytic infiltration, granular degenerative lesions was also observed. All these reports support the findings of present investigation.

Grossly, spleen was slightly enlarged in size with vascular congestion and haemorrhagic spots visible up to 14th days. Infiltration of macrophages and giant cells was seen with extinctic migratory routes. Regarding changes of spleen, Mondal (2002) noticed pale appearance and very slight spleenomegaly and Tudor (2004) also observed congestion and slight increase in volume in beginning of p.i. While histopathologically mesentric lymphnode showed early changes and strong reactivity due to infiltration of lymphocyte and hyperplasiated spleen follicle.

During examination of intestine, pin sized haemorrpoagic spots noticed in mucosa. However in intestinal section was found almost normal up to 2nd days but shortening of villi and inflamation were observed on 7th and 14th day p.i. Sharma and Bhatia (1983) also reported large number of *T. canis* larvae in jejunum and ileum portion of chicken. Hyat and Hyat (1984) also recovered larvae in intestine, and observed development not proceed beyond 2nd stage. However lymphatic vessels, larval peritoneal cavity and vasular system followed through actual larval penetration in intestinal blood vessels was not evident. Abo-Shehada *et al.* (1984-85) studied that *T. canis* larvae found prefered site for penetration at the base of crypts of leiberkhun followed by routes in lamina propria and tunica muscularis. Visible modification observed by Tudor *et al.* (2004) in intestine of rabbit infected with *T. canis* were haemorrhages in mucous membrane especially in ileum and caecal level due to mechanical action of larvae and their routes

through median integument along with intestinal lumen. Microscopic changes revealed with shortening of villi and local inflammation, which are also visualized in present result.

There was no major macroscopic change noticed in Skeletal muscles except certain migratory routes surrounded by eosinophils and macrophages. Mild granuloma was also observed in recovered tissue from larval migration. Tudor (2003-04) and Tudor *et al.* (2004) was unable to found any visible microscopic modification in striated musculature in guinea pigs or rabbit.

Concluding the present results, it can be suggested that rabbit was acted as unspecified/paratenic host for *T. canis* and *T. vitulorum.* In present study, larval migration was visualised in liver, lungs, kidneys, spleen, intestine and muscle on different days of observation which revealed intensity of lesions in different levels. The number of isolated larvae from liver and lungs showed highest reactivity level in those tissues during both infections. While kidney, spleen, intestine and musculature were less targeted organs by these larvae. In the present investigation, the migration of both *T. canis* and *T. vituloram* was somewhat identical. In most of these organs, resolution through migratory larvae observed after 21 day p.i. except in musculature.

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CHAPTER - VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The study of nematodes *Toxocara vitulorum* and *Toxocara canis*, the parasites from the small intestine of bovine and dogs are not only important for the veterinary science but also for human medicine and dairy industry. *Toxocara canis* is responsible for visceral larval migrans syndrome in human beings. Many animals also could become paratenic hosts after accidental ingestion of embryonated egg of *Toxocara canis* and *Toxocara vitulorum*. The larvae migration in all tissues of paratenic host organism determines morphological and haematological changes. For realizing the aim of present investigation to study the "Migratory behaviour of ascarid larvae with subsequent pathological changes in rabbit" was conducted in order to observed action of larvae migration in tissues at various post observation days on inoculation of 5000 embryonated eggs of *T. vitulorum* and *T. canis* in rabbits and also the evaluation of parasitic action on haematological characteristics.

Over all prevalence of toxocariosis in bovine calves and pups was studied through examination of 169 and 147 fecal samples collected from Patna and surrounding areas. The percentage of infection recorded was 80.47% in bovine calves while 42.17%, local pup population found infected with toxocariosis.

The effect of sex on adult worms recovered from buffalo calves (*Toxocara vitulorum*) and pups (*Toxocara canis*) was found non-significant.

To study the migratory behaviour of *Toxocara canis* and *Toxocara vitulorum* larvae in rabbits, 5000 embryonated eggs were inoculated orally in different groups of rabbits and haematological changes were observed and compared with control (healthy) group of rabbit on day 2nd, 7th, 14th, 21st and 30th day of post infection. Larval recovery, gross and histopathlogical changes in liver, lungs, kidney, spleen and skeletal muscles were noticed on similar days of observation.

In case of rabbits infected with *T. vitulorum*, there was evidence of haemoglobunaemia, leucocytosis, eosinophilia found throughout the course of investigation Besides this a significant decrease in packed cell volume, lymphocyte and neutrophil values than the control group was observed up to day 21 post infection. No significant difference was observed in mococyte percentage during the whole period of experimental period. Similar trend of results were also recorded in case of rabbits infected with *T. canis* but these changes were more pronounced than *T. vitulorum* infection.

The studies on migratory behaviour of larvae in term of larval recovery of *T. vitulorum* in different organs of rabbit revealed maximum larval recovery in liver followed by in lungs, intestinal content and skeletal muscles while larval counting was noted nil during examination of kidney and spleen

samples. However overall maximum larval recovery was recorded on day 2 post infection and thereafter a gradual decline in larval counting observed but organ wise highest larval count was noted in liver, lung, skeletal muscle and intestinal content on day 2nd, 7th and 30th of p.i. respectively.

While the recovery of *T. canis* larvae in different organs of rabbit revealed that, larval counting was maximum (377.6) in liver on day 2 p.i., 95.2 in intestinal content on day 7 post infection.

It was noticed that trend of migratory behaviour was almost similar both in case of *T. canis* and *T. vitulorum* infection in rabbits, lungs and liver were found most succeptible and target organs for larval invasion.

Histopathologically, Liver, Lungs, Kidney, spleen and muscles of rabbit experimentally infected with *T. vitulorum* and *T. canis* revealed migrating tracts filled with haemorrhagic content, cellular infiltration along the tracts. Some degenerative changes in the parenchyma from tissue exhibited in the early stage of infection and section of larvae were also present in tissue sections. As the infection progressed, the cellular infiltration increased and there was granuloma formation consisting of lymphocytes, eosinophil, macrophages and few giant cells. Resolution of migratory routes and regeneration of tissues were observed after day 21 p.i. in all the tissues except musculature.

From the results it was evident that, following oral the oral administration of *T. canis* or *T. vitulorum* embryonated eggs, the larvae migrate through liver and lungs and invade the visceral organs, causing visceral phase in early stage and then migrate to muscles where they accumulated and lodge causing myotropic phase in later stage of infection. Thus it concluded that the visceral larval migrans (VLM) can be studied well in the rabbit model system.

CONCLUSION :

(i) The over all incidence of *T. vitulorum* and *T. canis* was 80.47 and 42.17% recorded in local bovine calves and pups respectively in Patna and its Surrounding area.

(ii) The average worm load was observed 4.80 and 13.80 for *T. canis* and *T. vitulorum* respectively and the effect of sex was found non-significant among recovered worms.

(iii) Considerable migratory changes observed on post inoculation of 5000 eggs/ml in one experimental rabbit both in case of *T. canis* and *T. vitulorum.*

(iv) Maximum larvae migrated through liver and lungs, caused cellular changes and disorganization of organs architecture and made their routes through other visceral organs including kidney, spleen and then migrated to muscles, in both the cases.

(v) Haematological changes due to migratory larvae caused, significant decrease in haemoglobin percentage as well as pack cell volume throughout the experimental period. Marked leucocytosis, lymphopaenia and neutropania, were also observed up to mid-way of infection and there by increasing tendency observed till end of the experiment. Eosinophilia was the most remarkable feature during the whole experimental period. The trend of haematological changes were same, both in case of migration of *T. vitulourm* and *T. canis* but the changes were more pronounced in case of *T. canis.*

(vi) During the gross examination of liver, lungs, kidney, spleen and skeletol muscles, marked differences were noticed in morphology of the organs. Haemorrhagic foci were also evident in different organs.

(vii) Histopathologicaly, changes in liver were degenerative in nature, where as in lungs, congestion, emphysema, pneumonia and atelectasis were marked. In all the organs haemorrhagic migratory routes were infilterd with lymphocyte, eosinophil, macrophages and giant cells.

(viii) Any appreciable difference was not marked in the morphology of both migratory larvae, which remained in the L2 stage throughout the experimental period in its paratenic host 'Rabbit'.

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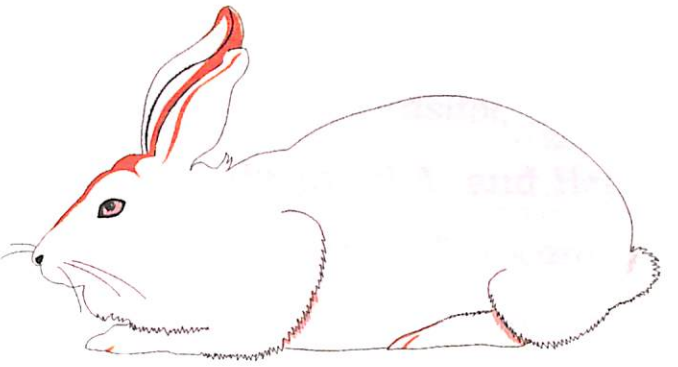

CHAPTER - VII

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