ASPECTS OF THE MORPHOLOGY, LIFE CYCLE AND EPIDEMIOLOGY OF *TOXOCARA* SPECIES AND *TOXASCARIS LEONINA*

By

GIRMA MEKETE TEKELE

Dissertation Submitted in the fulfillment of the requirements for the degree of

Master of Sciences in the Faculty of Natural Sciences

Department of Zoology and Entomology

University of the Free State

Supervisor: Prof. Dawie J. Kok

Co-supervisor: Prof. Linda Basson

March 2003

DECLARATION

I hereby declare that this dissertation prepared for the degree of Master of Sciences, which was submitted by me to the University of the Free State, is my own original work and has not previously in its entirety or in part been submitted to any other university. All sources of materials and financial assistance used for the dissertation have been duly acknowledged. I also agree that the University of the Free State has the sole right to the publication of this dissertation.

Signed on _____ 2003 at the University of the Free State, Bloemfontein, South Africa.

Name: Girma Mekete Tekele Signature: _____

ACKNOWLEDGMENTS

I would like to convey my deepest and sincere gratitude whole-heartedly to the following people and institutions.

- Prof. Dawie J. Kok (Major supervisor) and Prof. Linda Basson (co-supervisor) for their keen interest in my research project, planning and execution. They deserve special thanks for their thoughtfulness, unreserved encouragement, genuine and sound suggestions, constructive discussions, extensive comments, valuable support and guidance all through planning and execution of my research to the writing of the final paper.
- Jimma University and School of Medical Laboratory Technology, Jimma, Ethiopia for granting me this study opportunity.
- Ireland Aid Ethiopia for financial assistance for my study.
- University of the Free state, Department of Zoology and Entomology, Bloemfontein, South Africa for giving me the opportunity, for the use of departmental facilities and equipment, and support during my study.
- Prof. J.G. Van As and Dr. Liesl L. Van As for their advice and help for the use of departmental facilities and equipment.
- The staff of ClinVet International (PLY), Bloemfontein, Republic of South Africa, for the use of departmental facilities, collection and provision of specimens.
- The staff of the Animal Unit, University of the Free State, in particular Dr. Freek Potgieter for his assistance in arranging, infecting and the follow-up of the infected mice and for the use of the laboratory facilities.

- The staff of Medical Laboratory Technology, Jimma University, in particular to Wondwossen Teresa, for his support in the epidemiological study conducted in Ethiopia.
- Research and publication office, Jimma University.
- Fellow parasitology students: Candice Jansen van Rensburg, Cecile C. Reed, Nico G. Grobler, Jonie Venter and Johann Van As in the Department of Zoology and Entomology, Free State University, for their support and assistance.
- Mrs. Susan Vermeulen for teaching me the Afrikaans language.
- My mother Beyenech Lemma, my wife Tigist Tekle for their love, patience, constant inspiration, and encouragement throughout my study period.

Above all, I thank the **ALMIGHTY GOD**, in whom I always trust, for giving me patience and endurance to complete my study without whom none of this study would have been possible.

DEDICATION

This piece of my work is dedicated with love and respect to my mother Weyzero **Beyenech Lemma** and my late father Ato **Mekete Tekele** who provided me with the opportunity of education and appreciating its fruit. Also to my wife **Tigist Tekele** and our son **Abenezer Girma**.

Table of Contents

Contents **Page**

CHAPTER I

GENERAL INTRODUCTION AND LITERATURE OVERVIEW

The word helminth is derived from the Greek words *helmins* or *helminthos*, which means a worm. It usually refers to the flatworms (*Platyhelminthes*) and roundworms (*Nematoda*, formerly known as *Nemathelminthes*). The nematodes are unsegmented worms usually cylindrical and elongated in shape. Their body is covered with a cuticle. These are among the most abundant animals on earth. The majority of nematodes are free-living and a few are parasitic forms. There are about 16000 species of nematodes described and it is assumed that many more exist (Kassai 1999).

Toxocara canis (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902), are among the largest of intestinal nematodes of domestic pets. The name *Toxocara* Stiles, 1905 is derived from a combination of the Greek words *toxon* that means, "bow" and *kara* (*cara*) that means "head" (Grove 1990), denoting the shape of the anterior portion ("head") of the adult worms.

Toxocara canis, T. cati and *T. leonina* are roundworms or ascarids of dogs and/or cats. Adult ascarids infecting dogs and cats, have very close morphological similarities with each other and with those of other animals and humans. Their resemblance in external morphology, the occurrence of mixed infections in dogs and in cats, and accidental spurious infections in humans, create confusion in their identification and may lead to inappropriate diagnoses and unnecessary treatment.

According to Nichols (1956), prior to the recovery of *T. canis* larvae from the liver of a boy (Beaver, Snyder, Carrera, Dent and Lafferty 1952), the identification of an infection with larval nematodes in humans was made only by the association of the larvae with concomitant intestinal ascariasis and not by means of known larval morphology. Later more attention was given to the study of morphological features of larval stages of *Toxocara* species to distinguish these from larval stages of other nematodes such as *Ascaris lumbricoides* (Linnaeus, 1758), hookworms and *Strongyloides stercoralis* (Bavay, 1876) which can also infect humans. Eggs of ascarids are usually more or less spherical, subglobose or oval in shape. The eggs of different species can usually be distinguished on the bases of size, colour, shape, content, presence or absence of pits and ridges on the egg surface. However, eggs of *T. canis* and *T. cati* have very close morphological similarities and may not be reliably distinguished using simple light microscopic examination (Uga, Minami and Nagata 1996).

Toxocara canis and *T. cati* have a complex life cycle, but it is simple and direct in *Toxascaris leonina.* A wide range of vertebrate and invertebrate animals may serve as paratenic hosts. Besides having a high reproductive potential in which a female worm produces vast numbers of eggs, these nematodes also have larval stages that are well adapted in their survival and transmission to successive generations of hosts by means of transplacental and/or transmammary transmission enabling them to counteract the low probability of individual offspring success (Glickman and Schantz 1981). Rodents play a significant role in the life cycle of ascarid species, serving as paratenic hosts (Sprent 1953). The use of paratenic hosts in *Toxocara* spp. and *Toxascaris leonina* also ensures

the survival of the larval stages, assuring long periods of survival while avoiding the unfavourable external environment.

Human infection with *T. canis* and *T. cati* is known as toxocariasis and this is one of the most common zoonotic helminthiasis (Herrmann, Glickman, Schantz, Weston and Domanski 1985; Gillespie 1993, Robertson, Irwin, Lymbery and Thompson 2000 and Humbert, Niezborala, Salembier, Aubin, Piarroux, Buchet and Barale 2000). Humans may be infected from contaminated body parts, soil, fomites, vegetables, fruit (Romeu, Roig, Bada, Riera and Munoz 1991; and Espana, Serna, Rubio, Redondo and Quintanilla 1993) and ingestion of raw or undercooked meat of paratenic hosts containing the larval stages, such as eating raw snails (Romeu *et al*. 1991), chickens (Nagakura, Tchibana, Kaneda and Kato 1989) and rabbits (Sturchler, Weiss and Gassner 1990). However, contaminated soil is considered the major source of exposure to the infective eggs.

In humans, infection with *Toxocara* spp. causes visceral larval migrans (VLM), ocular larval migrans (OLM) and covert toxocariasis (Smith 1998 and Taylor, Keane, O'Connor, Mulvihill and Holland 1988). The primary cause of toxocariasis is *T. canis* and occasionally *T. cati* (Glickman & Schantz 1981). After Beaver *et al.* (1952) reported *Toxocara* larvae in the liver of a child, *Toxocara* spp. has been considered as one of the causative agents of visceral larval migrans. Visceral larval migrans is usually prevalent in children aged one to four years (Schantz, Meyer and Glickman 1978). Ocular larval migrans has been reported occasionally, but has the most serious consequence of partial or complete loss of vision (Borg and Woodruff 1973). Ocular larval migrans usually

occurs in children aged four to eight years (Searl, Moazed, Albert and Marcus 1981) and rarely in adults (Roth and Gleckman 1985).

According to Thompson, Bundy, Cooper and Schantz (1986), the transmission of *T. canis* is influenced by the prevalence of infection in the local dog population, size of the local dog population, standard of hygiene in the human population, and exposure related behaviour. Children are particularly exposed to risks of infection with *Toxocara* spp. eggs because of their pica habit, close contact with infected dogs and playing on contaminated playgrounds (Shimizu 1993). Besides these, poor personal hygiene (Agudelo, Villareal, Caceres, Lopez, Eljach, Ramirez, Hernandez and Corredor 1990; Magnaval, Michault, Calon and Charlet 1994), lack of parental education (Sadjjadi, Khosravi, Mehrabani and Orya 2000), occupation (Clemett, Williamson, Hidajat, Allardyce and Stewart 1987) and pet ownership (Arpino, Gattinara, Piergili and Curatolo 1990; Holland, O'Lorcain, Taylor, and Kelly 1995; Alonso, Stein, Chamorro and Bojanich 2001) are also indicated as risk factors to the exposure of ascarid infections.

Climatic conditions such as temperature, humidity (Glickman and Schantz 1981, Parsons 1987) and soil type are also factors that influence the survival of the eggs in the environment. However, eggs of ascarids are highly resistant and may survive for years in the environment (Borg and Woodruff 1973; Searl *et al*. 1981; Urquhart, Armour, Duncan and Jennings 1989; Gillespie, Pereira and Ramsay 1991). According to Glickman and Schantz (1981), the degree of soil contamination reflects the prevalence of ascarids in the dogs and density of the dog population in an area.

Surveys conducted in many parts of the world to determine the prevalence of *T. canis* in dogs have reported prevalence rates ranging from 2.9% in the Netherlands (Overgaauw 1997a) to 89% in Japan (Shimizu 1993). However, as a result of host factors, management of domestic pets and techniques used to examine faecal samples, wide ranges of prevalence rates were reported in many parts of the world. Usually high prevalence rates were reported from puppies, stray dogs and in dogs investigated during necropsies. A high prevalence of patent infection occurs in puppies and kittens that are most often and most heavily infected with *Toxocara* spp. and contribute most to the contamination of the soil in the environment.

Epidemiological surveys for *T. cati* showed prevalence rates of 2% in the Netherlands (Overgaauw and Boersema 1998a), 84% in Australia (Uga *et al*. 1996; Milstein and Goldsmid 1997) and 100% from wildcats in Greece (Papadopoulos, Himonas, Papazahariadou and Antoniadou-Sotiriadou 1997). It has been suggested that *Toxocara* spp. eggs were found more frequently in kittens (Urquhart *et al*. 1989) and in stray cats than in cats kept in households (Overgaauw 1997a; Overgaauw and Boersema 1998a). *Toxascaris leonina* has been reported from dogs with a prevalence of 0.2% in Ireland (O'Lorcain 1994) to 52.19% in Turkey (Doganay and Oge 1993).

Domestic animals are usually infected with a wide variety of parasites as a result of their confined habitat, leading to extremely dense parasitic concentrations in the soil (Roberts and Janovy 2000). Soil is the principal source of many parasitic helminths of humans and animals (Ciarmela, Minvielle, Lori and Basualdo 2002). Considerable environmental

contamination exists because of the high fecundity and long survival of eggs of *Toxocara* in the environment (Jordan, Mullins and Stebbins 1993). Puppies, stray dogs, stray cats and wild carnivores are considered as sources of soil contamination with their faeces, which may contain eggs of the ascarids (Shimizu 1993). Soil contaminated with *T. canis* embryonated eggs is the main source of infection of man (Radman, Archelli, Fonrouge, Del-V-Guardis and Linzitto 2000). Studies conducted in many parts of the world has identified *Toxocara* spp. eggs in 15% of soil samples from playgrounds in Dublin (O'Lorcain 1994) to 92% in sandpits of public parks in Japan (Uga 1993; Shimizu 1993; Abe and Yasukawa 1997). The common occurrence of the eggs of *Toxocara* species in the environment has a potential risk to the health of human beings, particularly to children (Shimizu 1993; Ruiz De Ybanez, Garijo, Goyena and Alonso 2000).

In studies of human toxocariasis, surveys conducted in many countries showed seroprevalence of 3.6% in Spain (Fenoy, Cuellar and Guillen 1996) to 92.8% (Magnaval *et al*. 1994). However, the prevalence of human toxocariasis is unknown because toxocariasis is not a reportable disease and the diagnosis is difficult (Glickman and Schantz 1981), as the larvae do not develop to adult worms in human beings (Schantz and Glickman 1979) and neither eggs nor adult worms are passed in human faeces. Prevalence of seropositivity was associated with lower socio-economic development (Herrmann *et al*. 1985), education of parents, puppy ownership (Worley, Green, Frothingham, Sturner, Walls, Pakalnis and Ellis 1984), intensity and duration of exposure, adherence to worming routines (Slovak 1984), number of dogs in the

community, prevalence of infection in the dogs, pattern of defaecation habit of domestic pets, and level of soil contamination (Glickman and Schantz 1981).

Diagnosis of patent *Toxocara* infections in domestic pets is obtained by faecal examination or the examination of stomach and intestinal contents for adult worms. Faecal specimens of domestic pets and soil samples can be investigated for parasites by direct microscopy, concentration techniques such as flotation or sedimentation, or a combination of them (Dunsmore, Thompson and Bates 1984; Ruiz De Ybanez *et al.* 2000; Oge and Oge 2000). Accurate or absolute diagnosis of toxocariasis in humans is based on the direct demonstration and identification of the larval stages in tissue biopsies (Raistrick and Hart 1976; Pollard, Jarrett, Hagler, Allain and Schantz 1979; Schantz and Glickman 1979; Searl *et al*. 1981; Wang, Huang, Chan, Preston and Chau 1983; Kielar 1983; Bruckner 1985; De Sauza and Nakashima 1995; Yoshida, Shirao, Asai, Nagase, Nakamura, Okazawa, Kondo, Takayanagi, Fujita and Akoa 1999). However, it is usually difficult to demonstrate larvae in tissue biopsies. As a result, the epidemiological prevalence of toxocariasis in humans has never been determined accurately (Pollard *et al*. 1979). Serologic tests are usually considered as the most appropriate way of diagnosis (Bruckner 1985; Lynch, Eddy, Hodgen, Lopez and Turner 1988; Nunes, Tundisi, Heinemann, Ogassawara and Richtzenhain 1999; Dubinsky, Akao, Reiterova and Konakova 2000; Yamasaki, Araki, Lim, Zasmy, Mak, Taib and Aoki 2000). Enzyme-linked immunosorbent assay (ELISA), using larval secretory antigens, has been reported to be very sensitive and specific for the diagnosis of toxocariasis in humans (De Savigny, Voller and Woodruff 1979).

Though ascarids of domestic pets are cosmopolitan (Overgaauw 1997b; Radman *et al*. 2000) and mainly widespread in the tropics and temperate regions (Schantz, Weis, Pollard and White 1980), a number of epidemiological surveys of domestic pets and soil samples were conducted in the developed or developing world. However, in Africa, particularly in Ethiopia, which is socio-economically poor, where there is no proper sanitation and a high prevalence of human parasitic infections, little attention was given to the parasites of domestic pets, which cause zoonotic health problems to man and economic losses as a result of death of the infected animals.

An increase in the awareness of the morphology, life cycle, epidemiology, pathology and laboratory diagnosis of infections caused by *Toxocara* species provides a better plan for the identification, prevention and control of infections transmitted by dogs and cats. Based on these considerations, the objectives of the this study were:

- To investigate the morphological aspects of the developmental stages of *T. canis*, *T. cati* and *T. leonina*.
- To determine the possible transplacental and transmammary modes of transmission of *T. canis, T. cati* and *T. leonina* in a paratenic host as a model.
- To investigate the transmission of *T. leonina* to the definitive hosts through experimentally infected tissues of a paratenic host.
- To determine the epidemiology of *T. canis, T. cati and T. leonina* and other intestinal parasites of domestic pets in Jimma, Southwestern Ethiopia.

Specific objectives are found at the respective chapters. This study provides a comprehensive and detailed investigation on the morphological aspects, life cycle and epidemiology of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina*. The morphological aspects and life cycle studies were conducted at the University of the Free State, Department of Zoology and Entomology, whereas the epidemiological survey was carried out in Jimma, Southwestern Ethiopia.

The layout of this dissertation is as follows:

For the convenience of giving complete information, there are abstracts, introductions, material and methods, results, discussions and conclusions for each study subject. **Chapter 2** deals with the morphological descriptions of *T. canis*, *T. cati* and *T. leonina*. In **Chapter 3**, the transplacental and transmammary mode of transmission of these parasites in a paratenic host is presented. In **Chapter 4**, the transmission of *T. leonina* to the definitive hosts through experimentally infected tissues of mice is presented. A study on the epidemiology of the three parasites in Jimma, southwestern Ethiopia is presented in **Chapter 5**. General conclusions and discussion of the study is presented in **Chapter 6**. The literature referred to in this dissertation are presented in **Chapter 7**. The overall study is summarised in **Chapter 8** (English) and **Chapter 9** (Afrikaans). Terms used in this study are defined in the **Appendix I**. Ethical committee permits for sample collection for the epidemiological study in Ethiopia and for the use of mice as experimental animals are attached in **Appendix II**.

CHAPTER II

MORPHOLOGICAL ASPECTS OF *TOXOCARA CANIS*, *TOXOCARA CATI* AND *TOXASCARIS LEONINA*

2.1 *ABSTRACT*

Roundworms of domestic pets have very close morphological similarities between the developmental stages. Both dogs and cats are infected with Toxocara Stiles, 1905 spp. and Toxascaris leonina (V. Linstow, 1902). Dogs are usually infected with Toxocara canis (Werner, 1782) and cats with Toxocara cati (Schrank, 1788). However, the occurrence of mixed infection with T. leonina, accidental cross- infections in dogs and cats with Toxocara spp. and accidental infection of man with immature or mature stages of Toxocara spp. cause confusion in the identification of the species of these parasites. The aim of this study was to describe the gross and detailed morphological aspects of the various developmental stages of T. canis, T. cati and T. leonina recovered from their definitive hosts and from infected mouse tissues using light and scanning electron microscopes. A detailed morphological study of these ascarids confirmed their gross morphological similarities and some general and fine structural difference between the three species. Toxocara canis differs from T. cati by having a larger size, longer and narrower, spear-shaped cervical alae in the adult worms, and a larger and grossly pitted egg. It differs from Toxocara leonina by its size, specific arrangements of papillae on the tail of the male worm and the digiti-form of the tail of the male worm. Unlike Toxocara canis and T. leonina, T. cati worms have short and wide, arrow-shaped cervical alae. Toxascaris leonina differs from Toxocara spp. by having a tail gradually tapering to a point, and a specific arrangement of caudal papillae in the male worm, absence of

oesophageal ventriculation and absence of surface ridges on the eggshell. The larval stages of the three parasites embryonated in the eggs and larval stages of Toxocara spp. recovered from tissues of infected mice were similar to the second-stage in the eggs. Unlike the larvae of Toxocara spp., larvae of T. leonina undergo development in the tissues of infected mice and differ from the larval stages recovered from the eggs. Unlike previous studies by other investigators, the present study has revealed that a female adult T. canis worm can attain a size of 27.5 cm and that a male Toxocara spp. has a median precloacal papilla.

Keywords: *Toxocara canis, Toxocara cati, Toxascaris leonina, morphology, light microscope, scanning electron microscope.*

2.2 INTRODUCTION

Accurate species identification of ascarids at any life cycle stage is a necessary prerequisite for studying life cycles, epidemiology, population biology and systematics, and is also central to the diagnosis of the infection (Zhu, Gasser, Chilton and Jacobs 2001). The three winged species of ascarids that infect dogs and cats belong to the genera *Toxocara* and *Toxascaris* (Sprent 1956, 1958 and 1959). They are bilaterally symmetrical, elongated, tapered at both ends and are morphologically closely similar. They have a complete digestive system with a mouth anteriorly, a gut and an anus/cloaca posteriorly. The mouth has a dorsal and two lateral lips. Usually the tail is curved in the male worm and tapers to a point in the female. The body is covered with a cuticle. These roundworms are dioecious and females are larger than the male worms. There are four juvenile stages and an adult stage in the life cycle, each separated from the preceding one by an ecdysis, or moulting of the cuticle. Some of the important morphological characteristics used for the identification and classifications of adult worms are the structure of the cuticle (e.g. alae and ridges), the number and distribution of papillae, the presence or absence of amphids and phasmids, the presence or absence of esophageal caeca (ventriculus) between the esophagus and intestine, the size and morphology of the male spicules and the position of the vulva (Roberts and Janovy 2000).

Difficulties in the identification of *Toxocara canis*, *T. cati* and *Toxascaris leonina* occur as a result of their gross morphological similarities (Okoshi and Usui 1967a; Morgan 2000), in the adult and larval stages. Moreover, mixed infections with *T. canis* and *T. leonina* in dogs, mixed infections with *T. cati* and *T. leonina* in cats and spurious

infections in dogs, cats and paratenic hosts, including humans, with mature or immature developmental stages, cause confusion in the identification of these roundworms.

Roundworms of domestic pets do have some morphological similarities with the human intestinal roundworm, *Ascaris lumbricoides*. There are reported cases of spurious infection of humans with adult *T. cati* worms (Von Reyn, Roberts, Owen and Beaver 1978; Eberhard and Alfano 1998), with adult *T. canis* worms (Bisseru, Woodruff and Hutchinson 1966), with juvenile stages of *T. canis* (Beaver *et al*. 1952; Wang *et al*. 1983; De Souza and Nakashima 1995) and spurious infection of dogs with eggs of *A. lumbricoides* (Seah, Hucal and Law 1975; Doganay and Oge 1993). *Toxocara* spp. and *T. leonina* can be distinguished from *A. lumbricoides* by their winged heads (cervical alae). However, this difference may be overlooked and this can lead to incorrect identifications (Beaver *et al*. 1952).

Knowledge of comparative morphological features of developmental stages of *Toxocara* spp. and *T. leonina* helps to simplify the diagnosis of these parasites and avoids unnecessary confusion in identifying the parasites in the definitive and/or in paratenic hosts. Against this background, the aim of the present study was to provide detailed macroscopic and microscopic morphological descriptions of eggs, larvae and adult developmental stages of *T. canis*, *T. cati* and *T. leonina* collected from domestic pets and from a paratenic host (mice).

2.3 MATERIAL AND METHODS

2.3.1 Study area and period

This morphological study was conducted at the Zoology and Entomology Department, University of the Free State and ClinVet International (Pty) Ltd, Bloemfontein, Republic of South Africa, from February to December 2002.

2.3.2 Collection of parasites

Adult worms

Adult worms of *Toxocara canis* (Fig. 2.1A), *T. cati* (Fig. 2.1B), and *Toxascaris leonina* (Fig. 2.1C) were obtained from dogs and cats during necropsies. The necropsies were part of efficacy studies, approved by the ClinVet ethics committee.

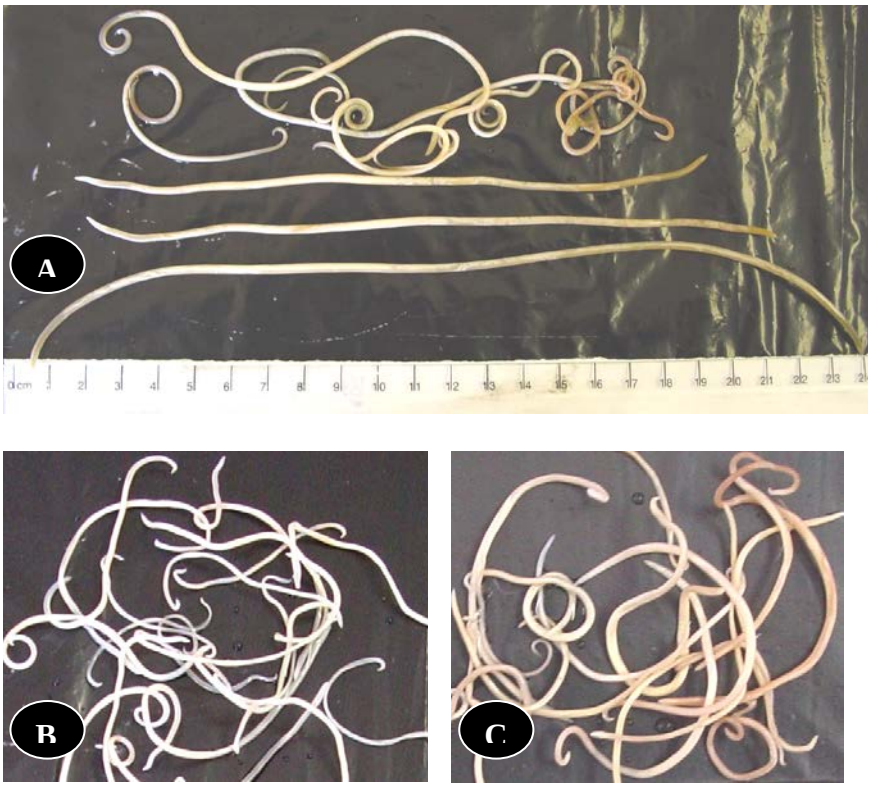


Figure 2.1. Adult male and female worms of *Toxocara canis* (Werner, 1782) (A), *Toxocara cati* (Schrank, 1788) (B), and *Toxascaris leonina* (V. Linstow, 1902) (C) collected from dogs and/or cats.

Eggs

Eggs of *Toxocara canis, T, cati* and *Toxascaris leonina* were collected from the uteri of adult female worms that were recovered during necropsies.

Larvae

Toxocara canis, T. cati and *Toxascaris leonina* larval stages were recovered from embryonated eggs that were collected from the uteri of adult female worms and cultured in 0.05 mol H_2SO_4 solution. After the eggs became embryonated, they were washed

repeatedly with tap water and transferred to a normal saline solution. The embryonated eggs were then incubated at 37^0C for 8-10 hours. Most eggs of *T. canis* and many eggs of *T. cati* hatched during this period. However, none of the embryonated eggs of *T. leonina* could be hatched using this method. Larvae of *T. leonina* were obtained from embryonated eggs by applying pressure to rupture the egg shells. This was done underneath a coverslip on a glass slide. Light tapping of the coverslip exerted sufficient pressure for the release of many larvae.

Larval stages of the three species were also recovered from mice experimentally infected for 7 - 34 days while conducting a study on the modes of transmission of these parasites in a paratenic host. Larval stages were collected and preserved in 5% formol saline solution (8.5 g NaCl, 50 ml absolute formaldehyde solution, and 950 ml distilled water).

2.3.3 Preparation and examination of parasite stages

2.3.3.1 Light and stereomicroscopy

Adult worms

Adult worms (Fig. 2.1A-C) collected from hosts during necropsies were properly washed with tap water before being examined. Size measurement (length) of the worms was taken from relaxed, live worms using a plastic ruler. The worms were then preserved in 5% formol saline solution, which ensured that they were fixed in an extended state (Soulsby 1982). A calibrated measuring eyepiece was used to measure the width at the midpoint of the worms under a stereo-microscope (Fig. 2.2). The sex of the worms was also determined with the aid of a stereo-microscope. Some of the smaller specimens were

studied microscopically as temporary mounts using lactophenol solution (20 ml pure glycerin, 10 ml lactic acid, 10 ml phenol crystals, and 10 ml distilled water) as a clearing agent.

Figure 2.2. Macroscopic examination and size measurement of adult specimens of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788), and *Toxascaris leonina* (V. Linstow, 1902).

Eggs

Properly cleaned non-embryonated and embryonated eggs were suspended in saline solution, transferred to a clean glass slide and covered with a coverslip (18 mm x 18 mm) for microscopic examination at various magnifications. Shape, size, colour, content and surface pitting of eggs were noted. Eggs size was measured after taking images of a calibrated scale and parasite eggs at the same magnification with a digital camera. Egg size was determined measuring the major axis as length and the minor axis as width. The size calculations were done using Scion image software (Scion Image 2000).

Larvae

The larval stages recovered from embryonated eggs and from the tissues of infected mice were washed with water and preserved in 5% formol saline solution for morphological studies with the aid of light microscopy. Size measurement was done as described for the eggs.

2.3.3.2 Scanning electron microscopy

Preserved eggs, larvae and anterior and posterior body parts of adult male and female ascarids were studied using scanning electron microscopy. All specimens were washed three times for 10 minutes with water, and then dehydrated through a series of graded ethanol concentrations as follows.

30% ethanol – 20 minutes

50% ethanol – 20 minutes

70% ethanol – 20 minutes

80% ethanol – 20 minutes

90% ethanol – 20 minutes

96% ethanol – 20 minutes, and

100% ethanol – 40 minutes.

Each of the above concentrations was renewed every ten minutes. The specimens were critical point dried and mounted on flat 12 mm aluminium stubs using a rapid drying varnish. Specimens were coated with gold and studied with the aid of a JEOL WinSEM JSM 6400 scanning electron microscope at 5kv or 10kv with the stage tilted at various

angles. Single or multiple specimen holders were used. Ilford Pan F films (50 ASA) were used for scanning electron micrographs.

2.3.4 Identification of eggs, larvae and adult worms

Identification of the developmental stages of each parasite was based on the description of Sprent (1956, 1958, 1959), Sprent and Barrett (1964), Soulsby (1982), Kassai (1999) and Roberts and Janovy (2000).

2.4 RESULTS

2.4.1 *Toxocara canis* (Werner, 1782)

Eggs

Eggs are more or less spherical (Fig. 2.3A-C) and dark brown in colour. When observed under the light microscope, the egg has a thick and corrugated shell (Fig. 2.3A & B). Egg surface pits and ridges look coarse and large when observed under the scanning electron microscope (Fig. 2.3C). In the unembryonated egg, the content of the ovum fills the egg and there is no open space between the ovum and egg shell (Fig. 2.3A). In embryonated eggs, the second-stage larvae were clearly seen (Fig. 2.3B) and some were motile. No operculum was found during scanning electron microscopic examination. The egg shell is torn and stretched when the larva escapes from the egg during hatching (Fig. 2.3D). Size measurement was done for 50 eggs. The eggs measured 83.55 (range 69.76 – 93.65) µm in length and 73.43 (65.78 – 80.67) µm in width (Table 2.1). The mean eggs size of *T. canis* is larger than that of *T. cati* but is smaller than that of *Toxascaris leonina* (Fig. 2.10; Table 2.1, 2.6, 2.11).

Table 2.1. Size measurements of *Toxocara canis* (Werner, 1782) eggs.

	Mean	Range	SD	N
Length (µm)	83.55	69.8 – 93.65	5.14	50
Width (µm)	73.43	65.78 – 80.67	3.67	50

SD = Standard deviation, N = Number

Second-stage larva

When an egg is excreted by the host, it is not embryonated but under favorable climatic conditions development proceeds to the first-stage larva, which moults inside the egg to become the infective second-stage larva. The second-stage larva of *T. canis* recovered from embryonated eggs (Fig. 2.3E) or from the tissues of infected mice (Fig 2.9A) has a cylindrical, stout or stumpy body. It is vigorously motile when it hatches. The body is covered with a cuticle (sheath), which remains from the first moult. In some larval stages, portions of the sheath were seen extending beyond the anterior and posterior ends of the body. The mouth is situated anteriorly and is directed slightly towards the dorsal side. The lips are not well defined. There are minute papillae on the lips. The lateral (longitudinal) alae are seen as refractile lines extending along the sides of the larva. Internal structures were poorly differentiated. The esophagus can be seen extending about one-third of the total length of the larva. The ventriculus of second-stage larvae separates the esophagus and the intestine (Fig 2.9A). The intestine is without an apparent lumen and is seen as an opaque granular mass in second-stage larvae recovered from the eggs (Fig. 2.3E). The primordial anal pore is found ventrally some distance from the tip of the tail. The tail is slightly curved dorsally and ends abruptly. The average length of 31 second-stage larvae recovered from embryonated eggs was 382.9 (range 197.8 – 428.6) µm and with an average width of 20.4 (range 16.6 – 24.1) µm at the mid-point of the body (Table 2.2). The larval stages recovered from the tissues of infected mice have comparable size measurements and are structurally very similar when observed under the microscope. The average length of 22 second-stage larvae recovered from tissues of

infected mice was 415.24 (range 384.82 – 437.95) µm in length and 22.39 (range 18.6 – 24.4) µm in width at the mid-point (Table 2.3).

Table 2.2. Size measurements of *Toxocara canis* (Werner, 1782) second-stage larvae recovered from embryonated eggs.

	Mean	Range	SD	N
Length (µm)	382.98	197.79 – 428.63	41.18	31
Width (µm)	20.38	16.63 – 24.08	1.78	31

SD = Standard deviation, N = Number

Table 2.3. Size measurements of *Toxocara canis* (Werner, 1782) second-stage larvae recovered from tissues of experimentally infected mice.

	Mean	Range	SD	N
Length (µm)	415.24	384.82 – 437.95	17.02	22
Width (µm)	22.39	18.6 – 24.4	1.87	22

SD = Standard deviation, N = Number

Adult worm

The *T. canis* adult worm (Fig. 2.1A) is cylindrical and white in color. In both female and male worms, the body is curved ventrally at the anterior end. The mouth is situated anteriorly and is surrounded by three lips, one dorsal and two ventro-lateral (Fig. 2.3F). The dorsal lip has two large papillae and two amphidial pores. Each subventral lip has a single large subventral papilla and a smaller lateral papilla. There is also an amphidial

pore on each subventral lip. There are numerous dentigerous ridges (denticles) arranged in one row on the external margin of each lip. The cervical alae (about 1.68 mm in length by 0.13 mm in width) extend some distance from the anterior extremity along the lateral margins (Fig. 2.3G). They are narrow, much longer than broad, and gradually taper posteriorly and anteriorly, giving a spear-shape or lanceolate appearance. There is a ventriculus at the posterior end of the esophagus and it distinctly separates the esophagus and the intestine (Fig. 2.4A). The vulva of the female worm opens as an oval transverse slit in the body wall (Fig. 2.4B) and is situated ventrally about a quarter of the body length from the anterior end. There are two phasmids (Fig. 2.4C), one on each side of the tail and each contains a minute pore at the center. The anal pore of the female worm opens as a transverse slit (Fig. 2.4D) and the tail tapers gradually to a point. The male worm ends with a narrow, digiti-form tail with a finger-like prolongation and has two equal sized spicules (about 0.13 mm long) (Fig. 2.4E). The two spicules are winged and appear thick and robust. There is a phasmid on each side of the tail and many papillae are arranged in rows with a specific pattern on the tail of the male worm (Fig. 2.4F). Two rows of 20-30 precloacal papillae, on each side of the tail of the male worm, are arranged in rows on the ventro-lateral surface. A single precloacal median papilla is situated near the cloaca. On each side of the digiti-form tail, there are two papillae on the ventro-lateral and two papillae on the lateral sides. Female worms are larger in size than male worms. Adult female worms measured 13.75 (6.1 – 27.50) cm in length and 0.18 (0.09 – 0.25) cm in width at the mid-point (Table 2.4). Adult male worms measured 9.09 (5.6 – 13.1) cm in length and 0.14 (0.06 – 0.20) cm in width at the mid-point (Table 2.5). The mean size measurements of adult female worm are larger than those of adult female

T. cati and *T. leonina* worms (Fig. 2.13). Similarly, the size measurements of adult male worm are larger than those of adult male *T. cat* and *T. leonina* (Fig. 2.14).

Table 2.4. Size measurements of *Toxocara canis* (Werner, 1782) adult females recovered from necropsies of experimentally infected dogs.

	Mean	Range	SD	N
Length (cm)	13.75	6.1 – 27.50	4.13	70
Width (cm)	0.18	0.09 – 0.25	O.04	70

SD = Standard deviation, N = Number

Table 2.5. Size measurements of *Toxocara canis* (Werner, 1782) adult males recovered from necropsies of experimentally infected dogs.

	Mean	Range	SD	N
Length (cm)	9.09	5.6 – 13.10	2.08	120
Width (cm)	0.14	0.06 – 0.20	0.03	120

SD = Standard deviation, N = Number

Figure 2.3. Light micrographs (A, B, E) and scanning electron micrographs (C, D, F, G) of *Toxocara canis* (Werner, 1782).

A. Non-embryonated egg showing the ovum and the corrugated egg shell. Scale bar: 20 μm.
B. Embryonated egg showing a second-stage larva within the egg. Scale bar: 20
C. Egg showing surface pits and ridges. Scale bar: 10 μm.
D. A second-stage larva escaping from an egg. Scale bar: 10 μm.
E. A second-stage larva recovered from an embryonated egg. Scale bar: 10 μm
F. Mouth of an adult worm showing a dorsal and two ventro-lateral lips and papillae on the lips. Scale bar: 100 μm.
G. Anterior body of an adult worm showing spear-shaped cervical alae. Scale bar: 1 mm.

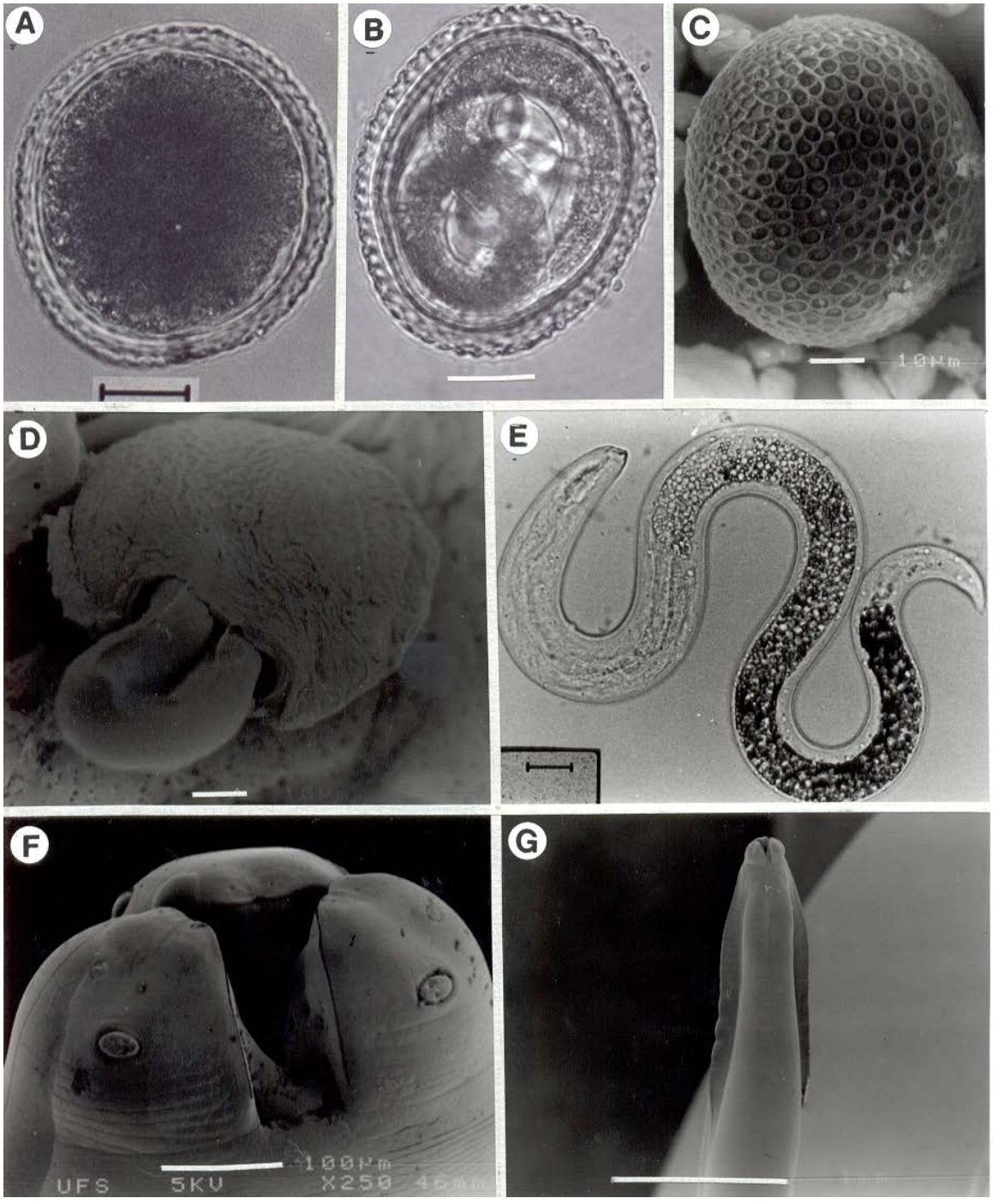
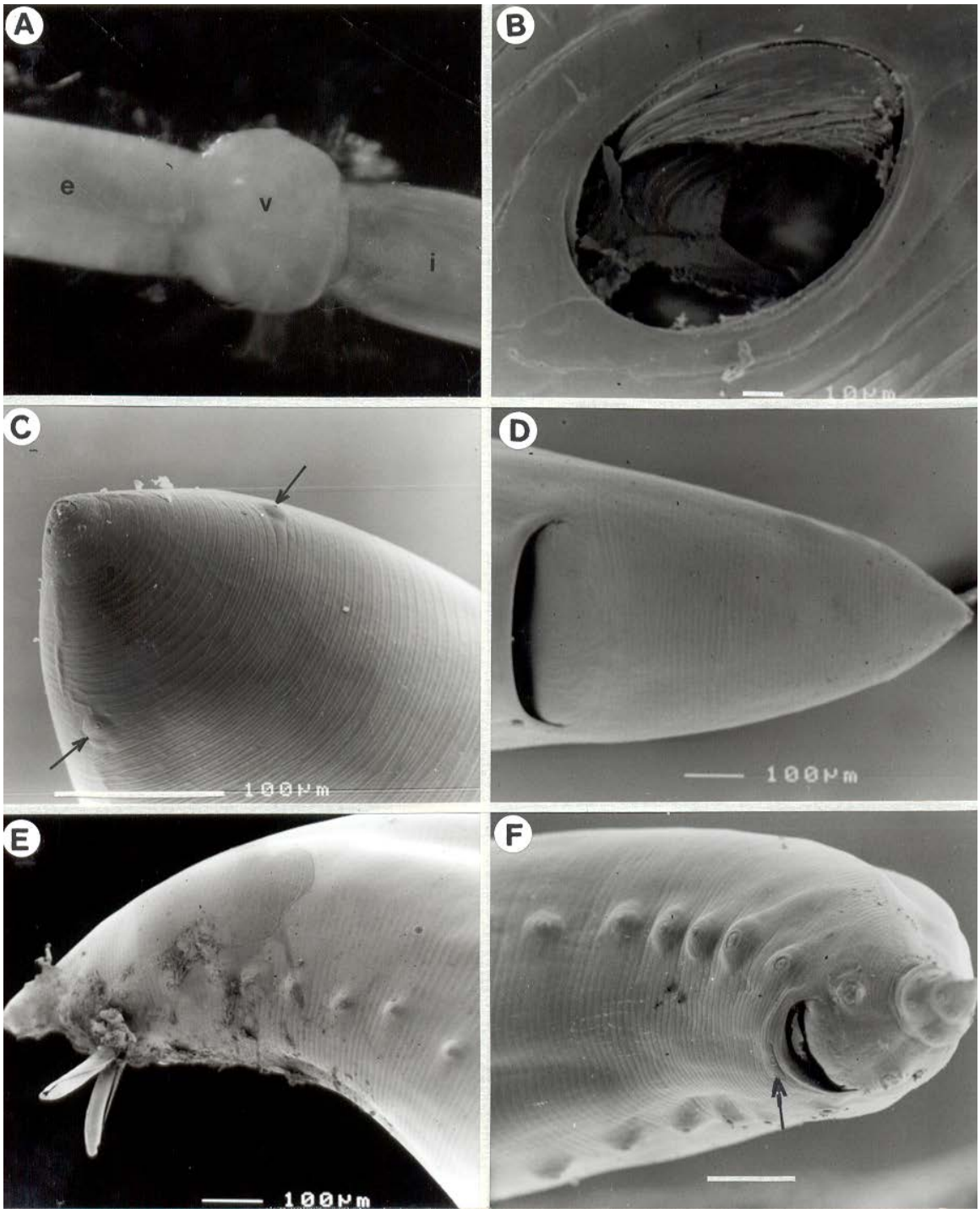

A
B
C
10µm
D
E
F
100µm
UFS 5KV X250 46mm
G

Figure 2.4. Light micrograph (A) and scanning electron micrographs (B, C, D, E, F) of adult *Toxocara canis* (Werner, 1782) .

A. Anterior parts of the gastrointestinal tract of an adult worm showing the esophagus (e), ventriculus (v), and intestine (i). 120X.
B. Vulva of adult female worm. Scale bar: 10 μm.
C. Tail of a female worm showing a phasmid (arrows) on each ventro-lateral side. Scale bar: 100 μm.
D. Tail of a female worm showing the anal opening and the tapering of the tail to a point. Scale bar: 100 μm.
E. The digiti-form tail of an adult male and its two spicules. Scale bar: 100 μm.
F. Tail of an adult male worm showing a median precloacal papilla (arrow) and the arrangement of caudal papillae. Scale bar: 100 μm.


A
e
v
i
B
100µm
C
100µm
D
100µm
E
100µm
F

2.4.2 *Toxocara cati* (Schrank, 1788)

Eggs

Eggs are more or less spherical in shape (Fig. 2.5A-C) but usually colourless and smaller in size than the eggs of *T. canis*. When observed under the light microscope, the egg has a corrugated egg shell and it appears to be slightly thinner than that of *T. canis* (Fig. 2.5A & B). When observed under the scanning electron microscope (Fig. 2.5C), the pits and ridges of the egg surface look smaller and more numerous in number than in the eggs of *T. canis*. In unembryonated eggs, the contents of the ovum fill the egg (Fig. 2.5A). In embryonated eggs, the second-stage larvae were clearly seen (Fig. 2.5B). Under light microscopic examination, a larva was observed hatching from an egg (Fig. 2.5D). The surface of the egg shell was examined for the presence of an operculum at the point where the larva escaped, and there was no structure indicating the presence of an operculum. Size measurements were done for 50 eggs. The eggs measured 73.07 (range 63.01 – 84.53) µm in length and 59.46 (range 53.20 – 72.26) µm in width (Table 2.6).

Table 2.6. Size measurements of *Toxocara cati* (Schrank, 1788) eggs.

	Mean	Range	SD	N
Length (µm)	73.07	63.01 – 84.53	4.82	50
Width (µm)	59.46	53.20 – 72.26	3.89	50

SD = Standard deviation, N = Number

Second-stage larva

The second-stage larva of *T. cati*, hatched from the egg (Fig. 2.5D) or released from the tissues of infected mice (Fig. 2.5E) is robust in shape and move vigorously. The second-stage larva released from the egg lies within the cuticular sheath of the first-stage larva and the cuticular sheath may be seen extending from the anterior and/or from the posterior end (Fig. 2.5D). The mouth is found anteriorly and directed towards the dorsal side as in the second-stage larva of *T. canis*. There are three lips each with a minute papilla. The lateral alae extend along almost the whole length of the body except to the anterior and posterior portions (Fig. 2.5E). In second-stage larvae released from the egg, the esophagus and the ventriculus are mostly indistinctly visible. The granular mass of the intestine and primordial anal pore are visible in the posterior part of the body. In some second-stage larvae, hatched from eggs and in most second-stage larvae recovered from tissues of infected mice, the ventriculus can be seen separating the esophagus and the intestine (Fig 2.9B). The primordial anal pore is found ventrally and the tail is usually curved and ends abruptly. Second-stage larvae recovered from embryonated eggs measured 394.12 (341.07 – 434.98) µm in length and 18.82 (15.03 – 21.44) µm in width at the mid-point (Table 2.7), while second-stage larvae recovered from infected mice tissues measured 389.38 (351.40 –425.0) µm in length and 19.0 (16.12 – 22.0) µm in width at the mid-point (Table 2.8).

Table 2.7. Size measurements of *T. cati* (Schrank, 1788) second-stage larvae recovered from embryonated eggs.

	Mean	Range	SD	N
Length (µm)	394.12	341.07 – 434.98	22.27	27
Width (µm)	18.82	15.03 – 21.44	1.66	27

SD = Standard deviation, N = Number

Table 2.8. Size measurements of *Toxocara cati* (Schrank, 1788) second-stage larvae recovered from tissues of experimentally infected mice.

	Mean	Range	SD	N
Length (µm)	389.38	351.40 – 425	22.19	24
Width (µm)	19.00	16.12 – 22.0	1.64	24

SD = Standard deviation, N = Number

Adult

Adult *Toxocara cati* worms are morphologically closely similar to *T. canis*. Like *T. canis*, adult *T. cati* worms (Fig. 2.1B) are also cylindrical in shape and white in color. In both sexes, the anterior end bends ventrally. The mouth is situated anteriorly and has three lips, one large dorsal and two smaller ventro-lateral lips (Fig. 2.5F). The dorsal lip has two large papillae and two amphidial pores. Each ventro-lateral lip has a single large papilla, a smaller lateral papilla and an amphidial pore. The dentigerous ridges are arranged on the margin of each lip. Unlike *T. canis*, the adult worms have short and broad cervical alae (1.5 mm in length by 0.25 mm in width) that are narrow anteriorly and

broad posteriorly giving the anterior tip of the body an appearance of arrow-shaped (Fig. 2.5F). There is a ventriculus (Fig. 2.6A) between the esophagus and the intestine. The vulva of the female which opens as an oval slit, is situated about one-fourth of the body length from the anterior end (Fig. 2.6B). The tail of the adult female worm has a paired phasmid laterally placed on the ventral side and it tapers gradually to a point (Fig. 2.6C). There is also a phasmid on each side of the tail of the male worm. The male worm, like *T. canis* has a digiti-form tail which bends to the ventral side (Fig. 2.6D). There are two equal sized winged spicules (Fig. 2.6D). They are longer (about 0.4 mm long) in size than the spicules of *T. canis,* look delicate and many of them were seen broken (Fig. 2.6E). In the male worm, 19 – 20 precloacal papillae, a single precloacal median papilla and 10 postcloacal papillae are arranged with a specific pattern (Fig. 2.6F) as in *T. canis.* Immediately posterior to the cloaca, a pair of papillae situated ventro-laterally; on each side of the digiti-form tail, as well as a pair of ventro-lateral and lateral papillae. Adult female worms measured 9.10 (5.80 – 11.70) cm in length and 0.14 (0.09 – 0.18) cm in width at the mid-point (Table 2.9). Adult male worms measured 6.60 (5.20 – 8.00) cm in length and 0.10 (0.07 – 0.13) cm in width at the mid-point (Table 2.10).

Table 2.9. Size measurements of *Toxocara cati* (Schrank, 1788) adult females recovered from necropsies of experimentally infected cats.

	Mean	Range	SD	N
Length (cm)	9.10	5.80 – 11.70	1.67	34
Width (cm)	0.14	0.09 – 0.18	0.025	34

SD = Standard deviation, N = Number

Table 2.10 Size measurements of *Toxocara cati* (Schrank, 1788) adult males recovered from necropsies of experimentally infected cats.

	Mean	Range	SD	N
Length (cm)	6.60	5.20 – 8.00	0.68	41
Width (cm)	0.11	0.07 – 0.13	0.014	41

SD = Standard deviation, N = Number

Figure 2.5. Light micrographs (A, B, D) and scanning electron micrographs (C, E, F) of *Toxocara cati* (Schrank, 1788).

A. Non-embryonated egg showing the ovum and corrugated egg shell. Scale bar: 16 μm.

B. Embryonated egg showing a second-stage larva within the egg. Scale bar: 15μm.

C. An egg showing surface pits and ridges. Scale bar: 10 μm.

D. A second-stage larva hatching from an egg. Scale bar: 1 μm.

E. A second-stage larva from the tissues of an infected mouse showing the lateral alae along the longitudinal margins. Scale bar: 10 μm.

F. The anterior portion of an adult worm showing the three lips and arrow-shaped cervical alae. Scale bar: 100μm.

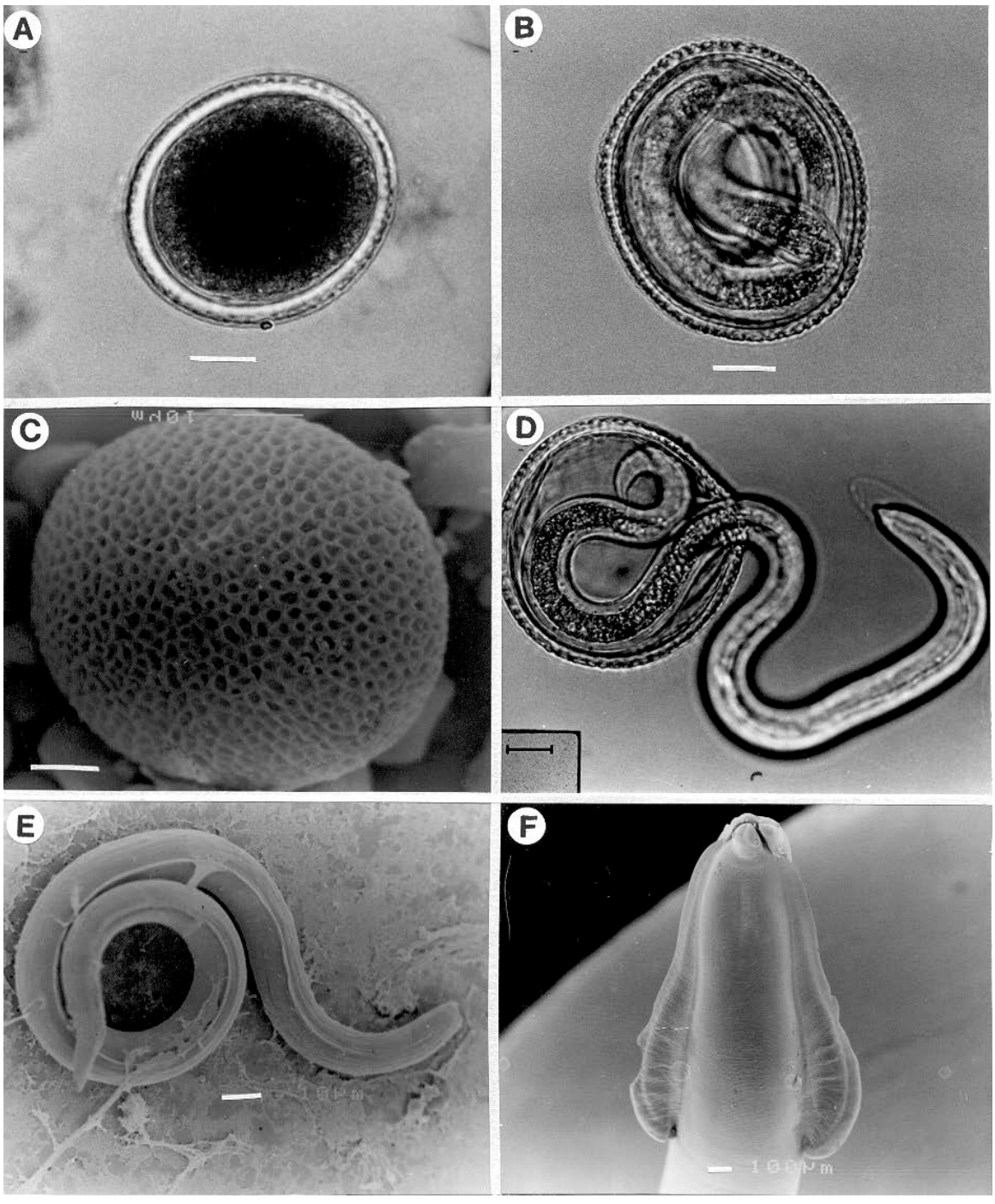
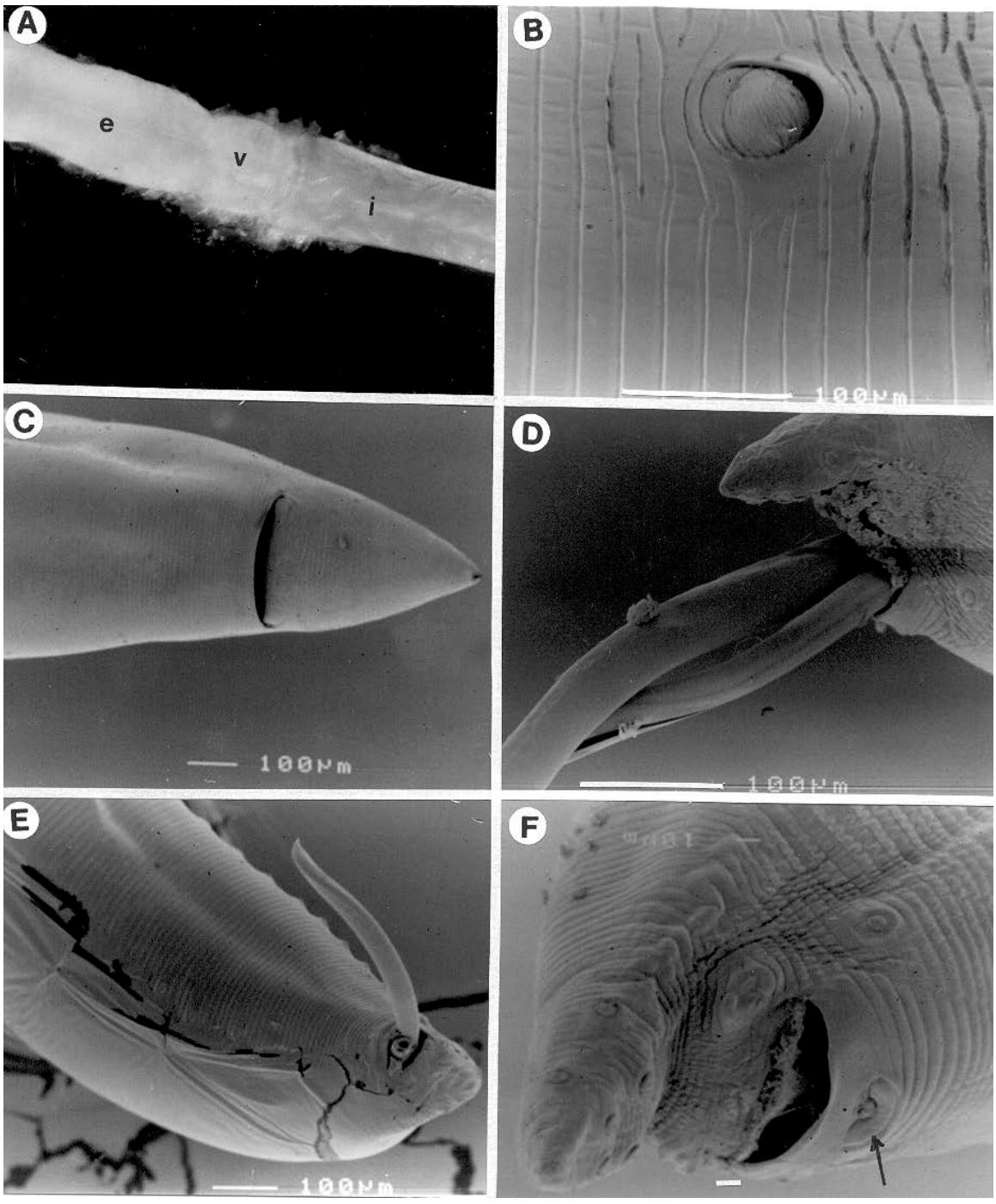

A
B
C
D
E
F

Figure 2.6. Light micrograph (A) and scanning electron micrographs (B, C, D, E, F) of adult *Toxocara cati* (Schrank, 1788).

A. Gastrointestinal tract of an adult worm showing the esophagus (e), ventriculus (v), and intestine (i). 120X.
B. Vulva of adult female worm. Scale bar: 100 μm.
C. Anal opening and tapering tail of a female worm. Scale bar: 100 μm.
D. The digiti-form tail and two spicules of a male worm. Scale bar: 100 μm.
E. Tail of a male worm of which one of the pair of spicules is completely broken off. Scale bar: 100 μm.
F. Tail of a male worm showing the presence of a median papilla (arrow) anterior to the cloaca and specific arrangements of postcloacal papillae. Scale bar: 10 μm.


A
e
v
i
B
100µm
C
100µm
D
100µm
E
100µm
F

2.4.3 *Toxascaris leonina* (V. Linstow, 1902)

Eggs

Toxascaris leonina eggs are usually oval in shape (Fig. 2.7A-C) and colourless. The egg shell appears to be thick and has a smooth surface unlike the eggs of *Toxocara* spp. Though light microscopy revealed no egg surface pits or ridges, a wrinkled surface was observed under the scanning electron microscope. No operculum was found during scanning electron microscopic examination. In the unembryonated egg, the ovum does not fill the egg and there is a space between the ovum and egg shell (Fig. 2.7A). The second-stage larva of *T. leonina* was clearly seen in the embryonated egg (2.7B) and was motile. A total of 50 eggs were measured. The average size measurement was 88.04 (range 79.06 – 99.07) µm in length and 70.12 (64.33 – 75.97) µm in width (Table 2.11).

Table 2.11. Size measurements of *Toxascaris leonina* (V. Linstow, 1902) eggs.

	Mean	Range	SD	N
Length (µm)	88.04	79.06 – 99.07	4.33	50
Width (µm)	70.12	64.33 – 75.97	2.74	50

SD = Standard deviation, N = Number

Larval stages

The second-stage larvae of *T. leonina* released from the eggs are stumpy in appearance (Fig. 2.7D) and were smaller than those of the second-stage larvae of *T. canis* and *T. cati* (Fig. 2.11). Second-stage larvae recovered from embryonated eggs (Fig. 2.7D) are smaller in size than those larvae recovered from the tissues of infected mice (Fig. 2.7E).

Larvae recovered from the eggs and from the tissues of infected mice moved less vigorously than those of *T. canis* and *T. cati,* usually lying straight. The body is covered with a sheath. The anterior part of the body is wider and gradually tapers posteriorly. The mouth has three lips that are more distinct in larvae recovered from the tissues of infected mice (Fig. 2.9C). Minute papillae could be seen on each lip and the elongation of the lips results in a prolongation of the anterior body of the larva. The lateral alae extend along almost the whole length of the body. The esophagus, intestine and anal pore are more distinct in larvae recovered from tissues of infected mice (Fig. 2.7E and Fig. 2.9C) than in those recovered from the eggs (Fig. 2.8D). The ventriculus between the esophagus and the intestine was seen in none of the second-stage larvae released from the eggs or in the larvae from the tissues of infected mice. A total of 26 second-stage larvae recovered from embryonated eggs measured 234.63 (203.7 – 288.1 µm) µm in length and 18.79 (15.4 – 22.6 µm) µm in width at the mid-point (Table 2.12), while 29 larval stages recovered from the tissues of infected mice measured 648.73 (434.59 – 790.78) µm in length and 30.55 (24.50 – 37.78) µm in width at the mid-point (Table 2.13). Larvae of *T. leonina* recovered from the tissues of infected mice were larger than those of the second-stage larvae of *T. canis* and *T. cati* recovered from the tissues of infected mice (Fig. 2.12).

Based on the differences in morphological features and size measurements, the larvae recovered from the tissues of infected mice were most probably third-stage larvae.

Table 2.12. Size measurements of *Toxascaris leonina* (V. Linstow, 1902) second-stage larvae recovered from embryonated eggs.

	Mean	Range	SD	N
Length (µm)	234.63	203.7 - 288.7	19.55	26
Width (µm)	18.79	15.4 – 22.6	1.91	26

SD = Standard deviation, N = Number

Table 2.13. Size measurements of possibly third-stage larvae *Toxascaris leonina* (V. Linstow, 1902) recovered from tissues of experimentally infected mice.

	Mean	Range	SD	N
Length (µm)	648.73	434.59 – 790.78	103.04	29
Width (µm)	30.55	24.50 – 37.78	3.97	29

SD = Standard deviation, N = Number

Adult

Adult *T. leonina* worms are cylindrical in shape (Fig. 2.1C) and closely similar to the adults of *Toxocara* spp. They are white to pinkish in color and their bodies tend to flex dorsally. Like adult *Toxocara* spp., the mouth is surrounded by three lips, one dorsal and two ventro-lateral (Fig. 2.7F). The dorsal lip has two large papillae and two amphidial pores. A single large subventral papilla, a smaller lateral papilla and an amphidial pore are present on each subventral lip. The papillae and amphidial pores are seen distinctly during scanning electron microscopic examination (Fig. 2.7F). There are numerous dentigerous ridges arranged in a row on the external margins of each lip. Like *T. canis*,

adult worms have long and narrow cervical alae (about 1.64 mm in length by 0.1 mm in width) that taper gradually anteriorly and posteriorly into the body giving the body a spear-shaped appearance (Fig. 2.7G). There is no ventriculus between the esophagus and the intestine (Fig. 2.7H), however, the junction between the esophagus and the intestine is seen clearly. The vulva of the female worm (Fig. 2.8A) is situated mid-ventrally one-third of the body length from the anterior end and a phasmid (Fig. 2.8B) is seen ventro-laterally on each side of the tail. The tail of the female worm tapers gradually to a point (Fig. 2.8C) as does the tail of the male worm (Fig. 2.8D). It is not digiti-form as in *Toxocara* spp. males. There are 25 to 32 pairs of ventro-laterally placed precloacal papillae, two pairs of papillae on each side of the cloaca and five postcloacal papillae on each side of the tail of the adult male worm (Fig. 2.8E). The positional arrangements of the postcloacal papillae are very specific. As shown in Figure 2.8F, the sequential arrangements of the papillae from the cloaca to the posterior end of the tail are; a pair of papillae on each side of the cloaca, a pair of postcloaca lateral papillae on each side of the tail, a subventral papilla on each side of the tail, and two papillae on each side of the tail that divert towards the subdorsal side. A phasmid is present on each side of the tail. A median precloacal papilla and caudal alae were not observed in the adult male worms. Adult female worms measured 8.38 (5.20 – 11.10) cm in length and 0.16 (0.12 – 0.22) cm in width at the mid-point (Table 2.14). The male adult worms measured 6.18 (4.50 – 7.20) cm in length and 0.14 (0.12 – 0.16) cm in width at the mid-point (Table 2.15). The mean size measurement of the adult male worm is larger than the mean sizes of adult male *T. cati* and *T. leonina* (Fig. 2.14). The morphological similarities and differences

between the eggs, larvae and adult *T. canis*, *T. cati* and *T. leonina* are summarized in Table 2.16.

Table 2.14. Size measurements of *Toxascaris leonina* (V. Linstow, 1902) adult females recovered from necropsies of experimentally infected dogs.

	Mean	Range	SD	N
Length (cm)	8.39	5.20 – 11.10	1.32	20
Width (cm)	0.16	0.12 – 0.22	3.04	20

SD = Standard deviation, N = Number

Table 2.15. Size measurements of *Toxascaris leonina* (V. Linstow, 1902) adult males recovered from necropsies of experimentally infected dogs.

	Mean	Range	SD	N
Length (cm)	6.18	4.50 – 7.20	0.88	12
Width (cm)	0.14	0.12 – 0.16	0.13	12

SD = Standard deviation, N = Number

Figure 2.7. Light micrographs (A, B, D, E, H) and scanning electron micrographs (C, F, G) of *Toxascaris leonina* (V. Linstow, 1902).

A. Non-embryonated egg showing the ovum and smooth egg shell. 15 μm..
B. Embryonated egg showing a second-stage larva inside in the egg. 14 μm.
C. Egg with a wrinkled surface and absence of pits and ridges. Scale bar: 10 μm.
D. A second-stage larva recovered from an embryonated egg. Scale bar: 15 μm.
E. A second-stage larva recovered from the tissues of an infected mouse. Scale bar: 21μm.
F. Mouth of an adult worm showing a dorsal and two ventro-lateral lips and papillae on the lips. Scale bar: 10 μm.
G. Anterior body region of an adult worm showing spear-shaped cervical alae. Scale bar: 100 μm.
H. Gastrointestinal tract of an adult worm showing the esophagus (e), intestine (i) and absence of a ventriculus. Scale bar: 120X.

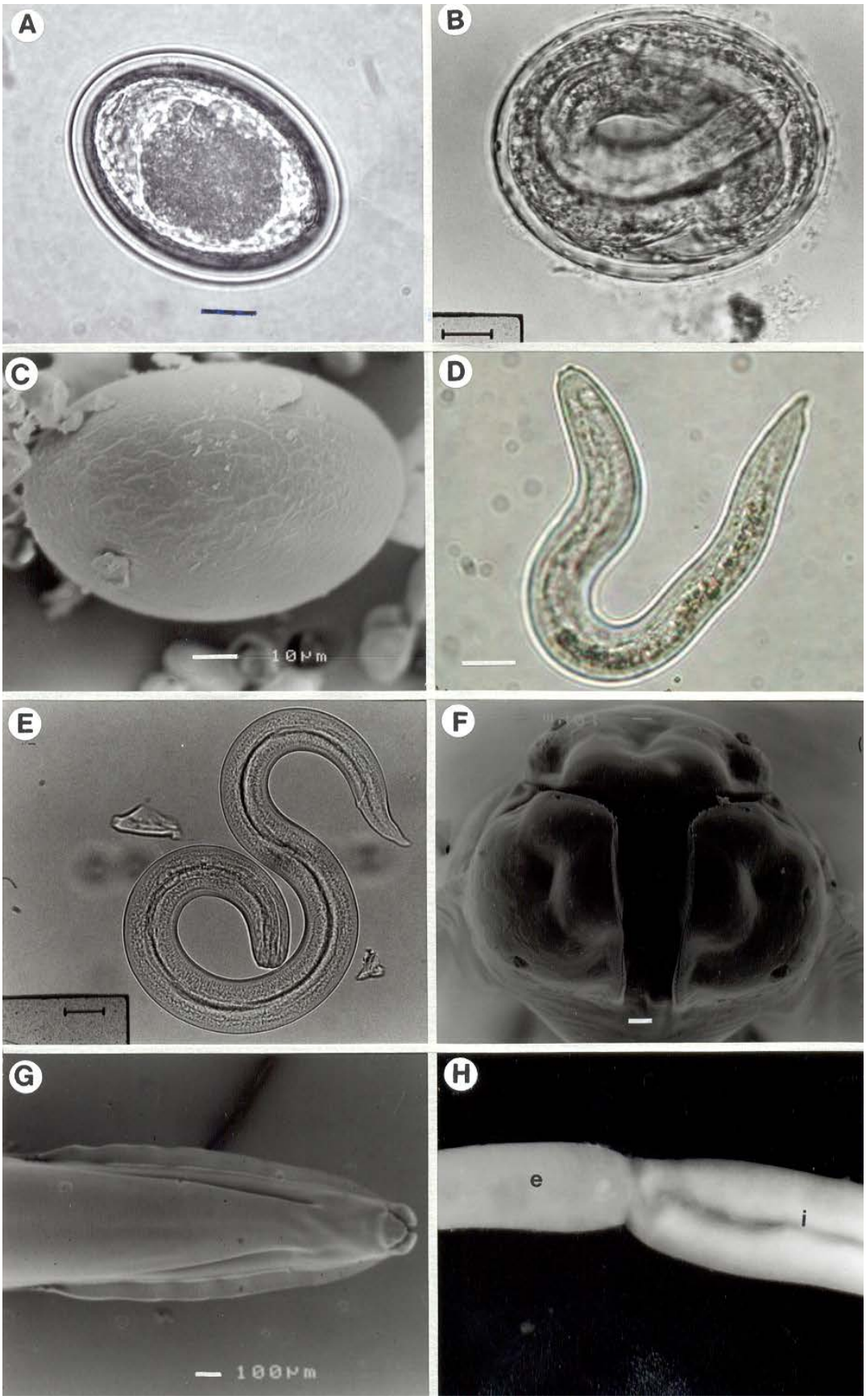
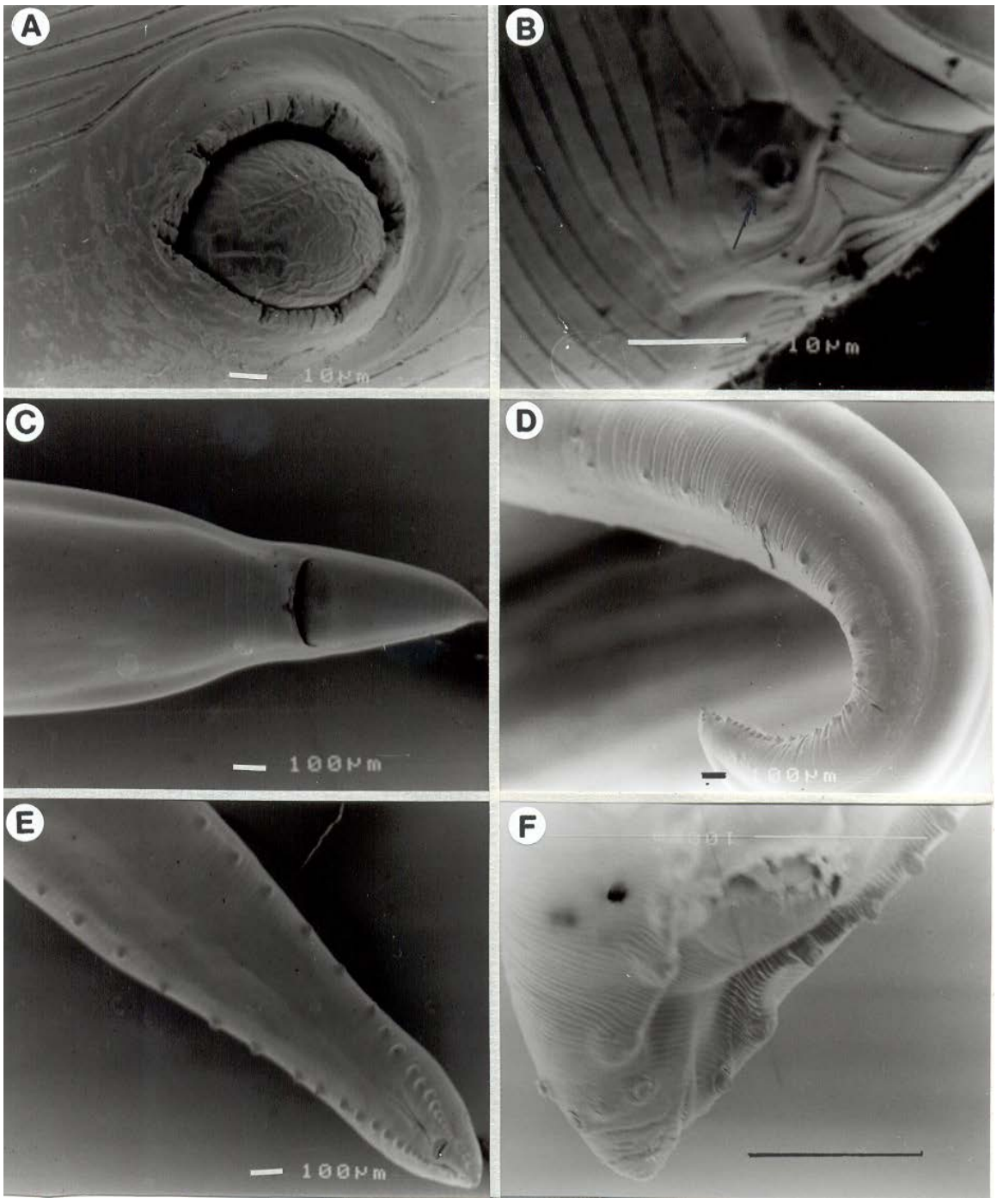

A
B
C
10μm
D
E
F
G
100μm
H
e
i

Figure 2.8. Scanning electron micrographs of *Toxascaris leonina* (V. Linstow, 1902).

A. Vulva of adult female worm showing an egg being laid. Scale bar: 10 μm.
B. A phasmid (arrow) on the lateral side of the tail of an adult female worm. Scale bar: 10 μm.
C. Anal opening and tapering tail of a female worm. Scale bar: 100 μm.
D. Tail of a male worm showing the gradual tapering of the tail to a point. Scale bar: 100 μm.
E. Tail of a male worm showing arrangement of precloacal and postcloacal papillae. Scale bar: 100 μm.
F. Tail of an adult male worm showing the arrangement of postcloacal papillae. Scale bar: 100 μm.


A
10μm
B
10μm
C
100μm
D
100μm
E
100μm
F

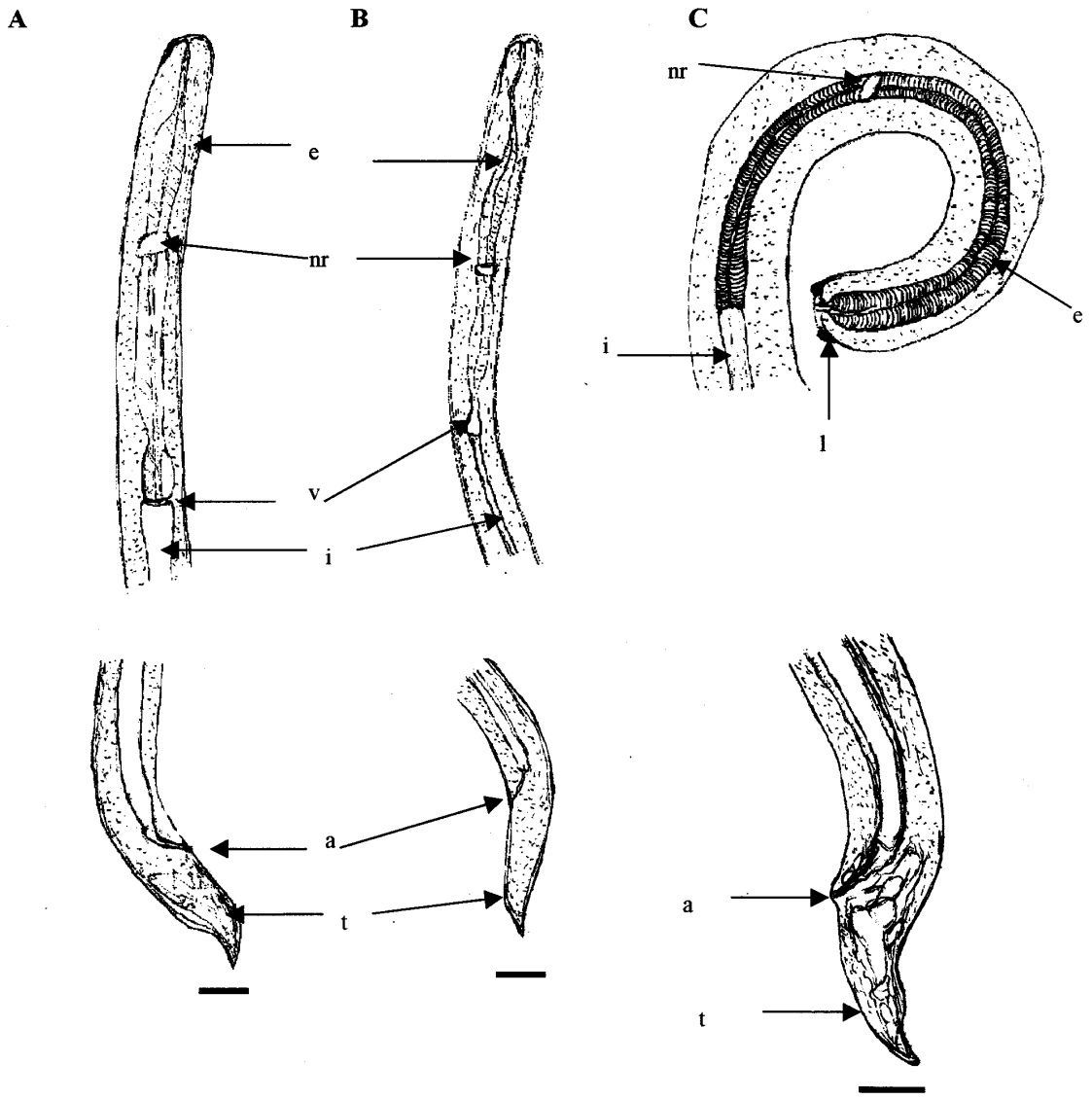


Figure 2.9. Microscope projection drawings of the anterior and posterior body parts of *Toxocara canis* (Werner, 1782) (A), *Toxocara cati* (Schrank, 1788) (B) and *Toxascaris leonina* (V. Linstow, 1902) (C) larvae recovered from tissues of infected mice. a = anal pore, e = esophagus, i = intestine, l = lip, nr = nerve ring, t= tail, v = ventriculus. Scale bar: A = 17 10μm; B and C = 19 μm.

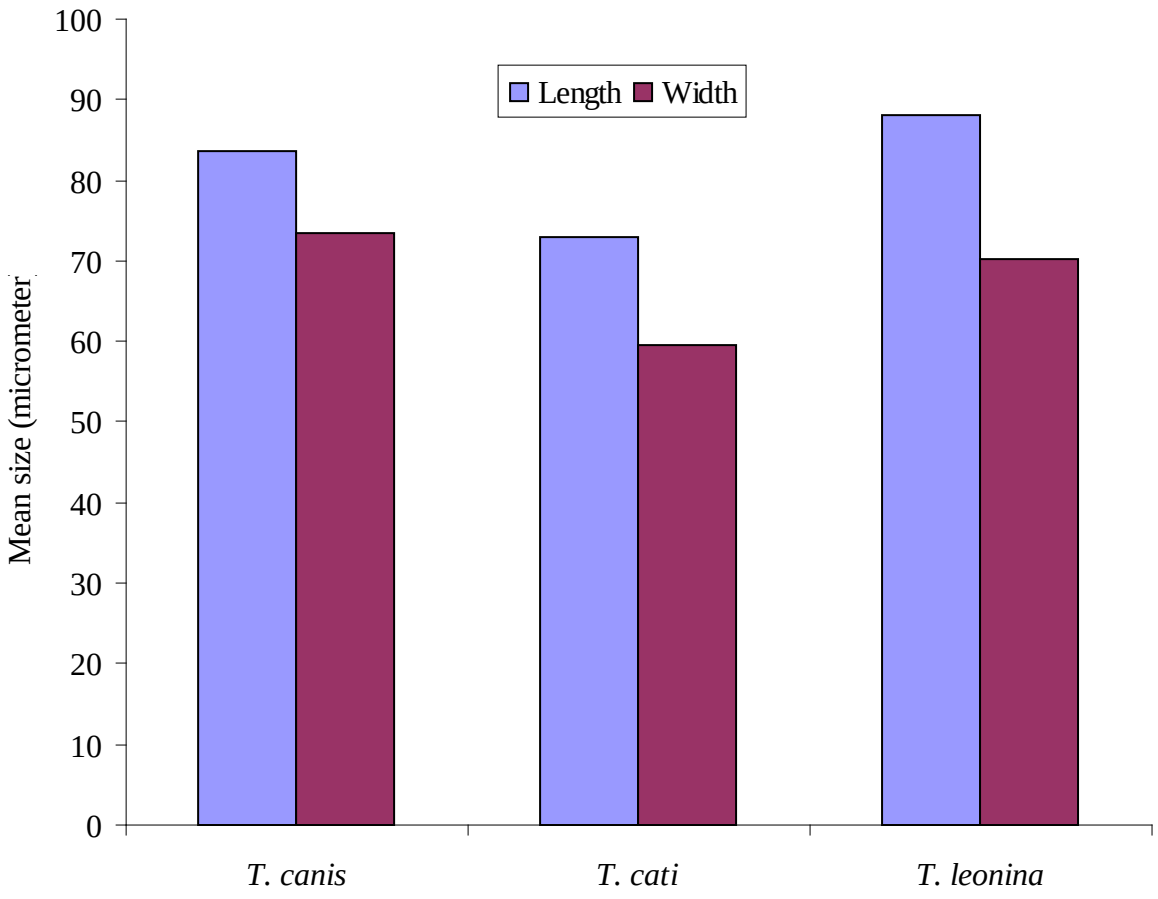


Figure 2.10. Mean size measurements of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788), and *Toxascaris leonina* (V. Linstow, 1902) eggs.

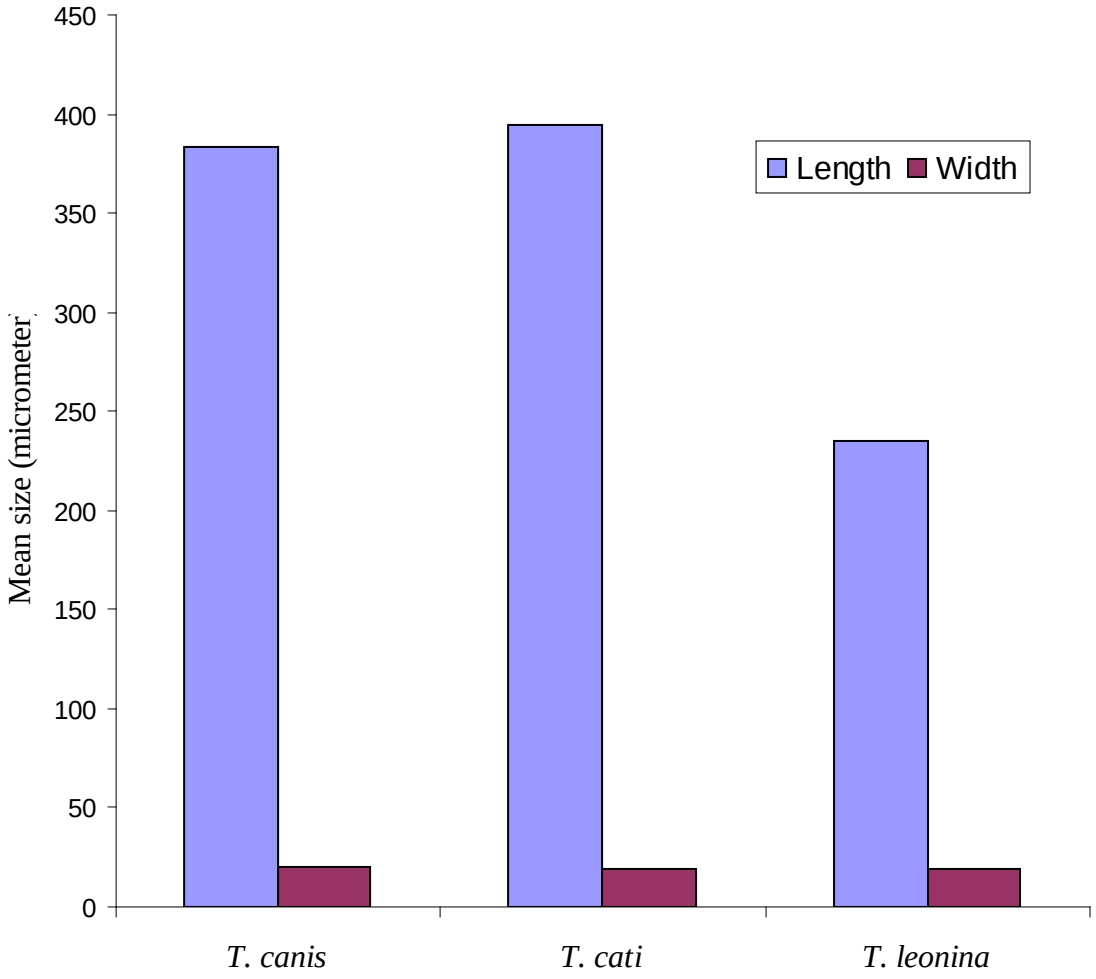


Figure 2.11. Mean size measurements of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) larvae recovered from embryonated eggs.

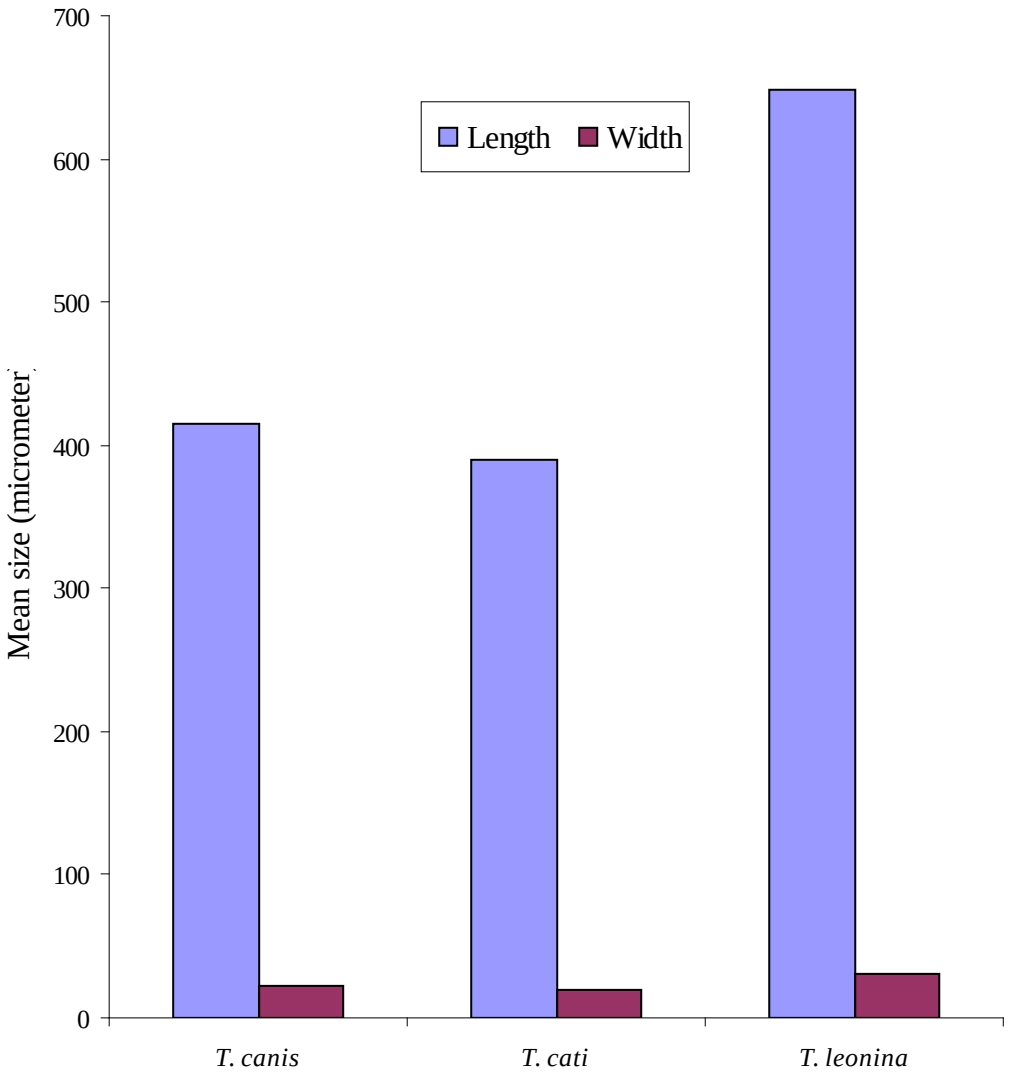


Figure 2.12. Mean size measurements of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) larvae recovered from tissues of infected mice.

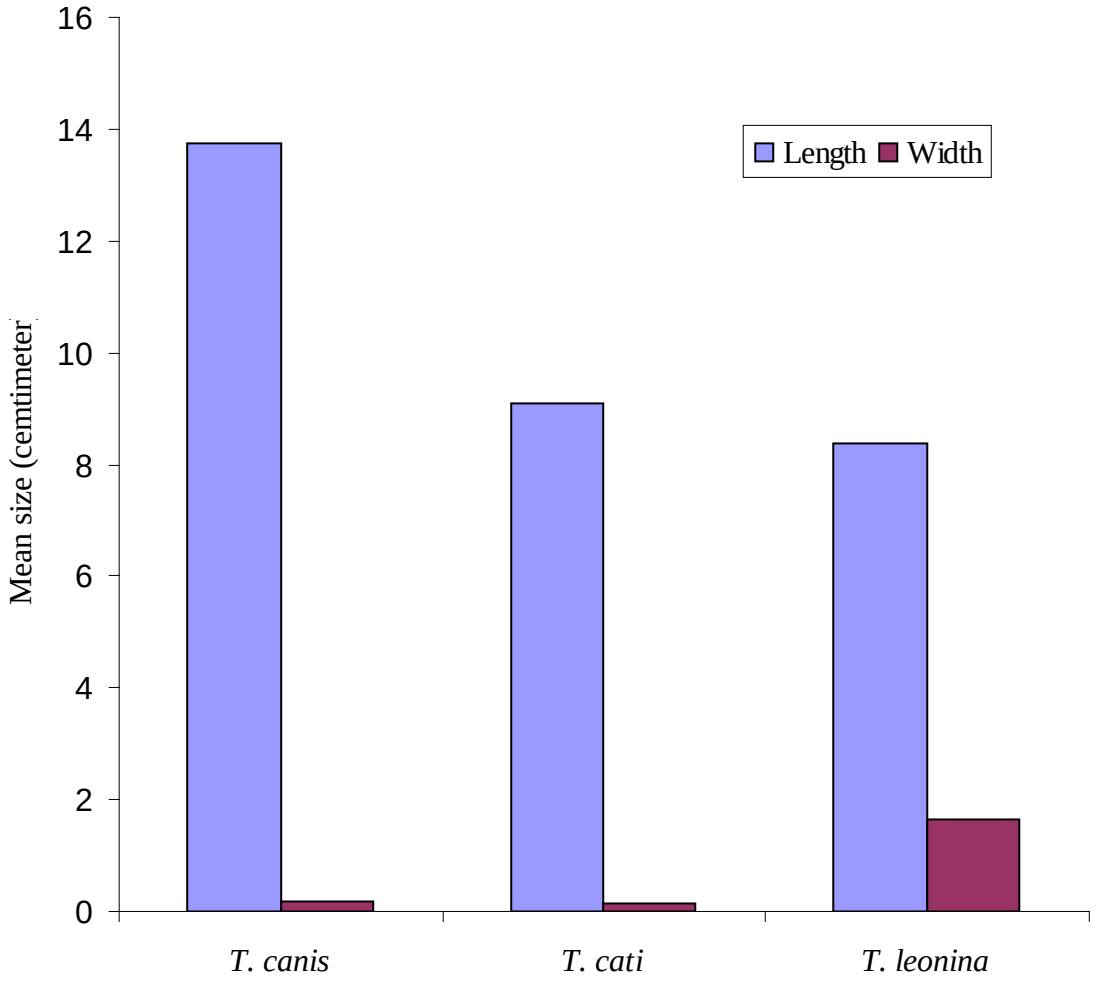


Figure 2.13. Mean size measurements of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) adult female worms.

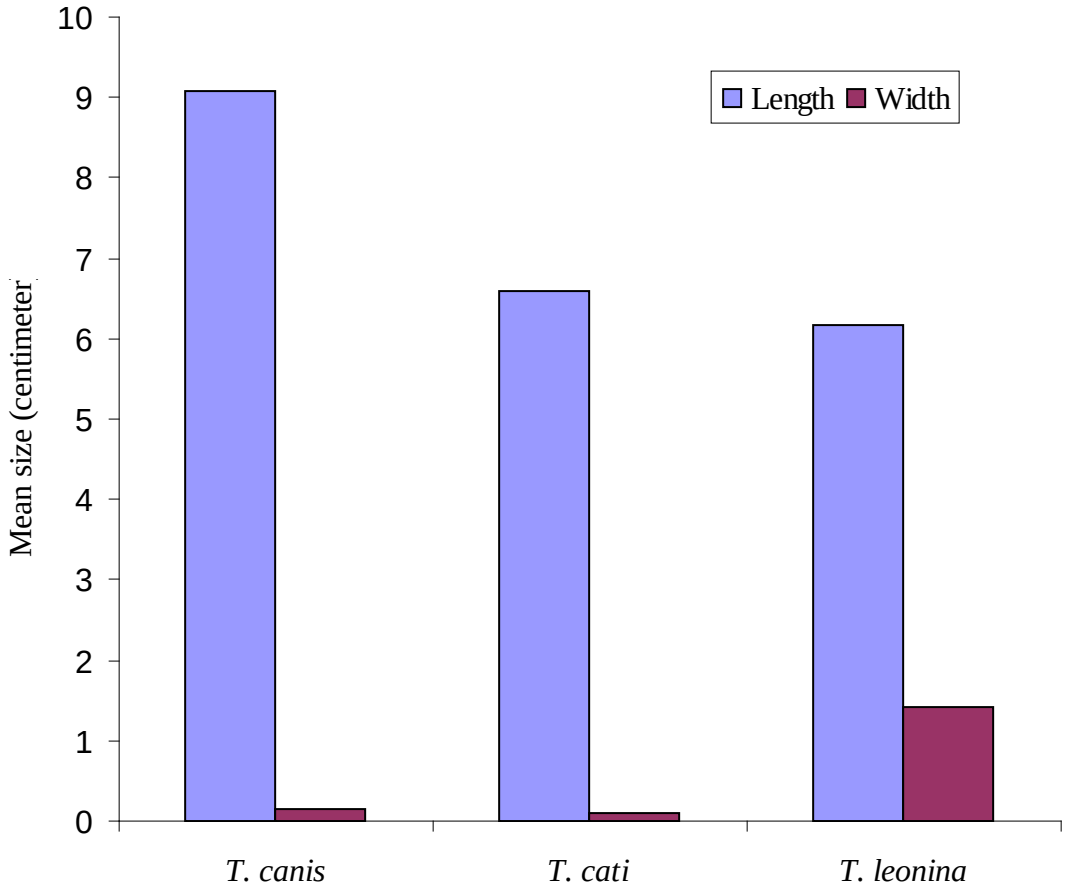


Figure 2.14. Mean size measurements of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) adult male worms.

Table 2.16. Morphological features of the developmental stages of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902).

Developmental stage	*Toxocara canis*	*Toxocara cati*	*Toxascaris leonina*	Remarks
Eggs	More or less spherical	More or less spherical	Usually oval	
	Thick and corrugated eggshell	Thin and corrugated eggshell	Smooth eggshell	
	Coarse and large egg pits and ridges	Fine and small egg pits and ridges	Has no egg pits and ridges	
	83.54 x 73.43 μm	73.07 x 59.46 μm	88.04 x 70.12 μm	
Larvae	Vigorously motile	Vigorously motile	Less vigorously motile	
Adults	Ventrally curved anteriorly	Ventrally curved anteriorly	Dorsally curved anteriorly	
	Spear-shaped cervical alae	Arrow-shaped cervical alae	Spear-shaped cervical alae	
	Has esophageal ventriculation	Has esophageal ventriculation	Has no esophageal ventriculation	

Female	13.75 x 0.19 cm	9.10 x 0.14 cm	8.39 x 0.16 cm	
	Vulva in the anterior quarter of the body	Vulva in the anterior quarter of the body	Vulva in the anterior quarter of the body	
male	9.09 x 0.14 cm	6.60 x 0.10 cm	6.18 x 0.14 cm	
	Digti-form tail	Digti-form tail	Tail gradually tapering to a point	Has specific pattern of arrangement
	20-30 pairs of precloacal papilla and 5 pairs of postcloacal papillae	19-20 precloacal papillae and five pairs postcloacal papillae	25-32 precloacal papillae and 5 pairs of postcloacal papillae	
	Has a precloacal median papilla	Has a precloacal median papilla	Has no precloacal median papilla	
	Has two thicker, winged spicules	Has two more delicate, winged spicules	Not seen in this study	

2.5 DISCUSSION AND CONCLUSIONS

Eggs

When observed under light microscopy, the eggs of *T. canis* and *T. cati* are very similar in their morphological features. However, it has been observed that most eggs of *T. cati* are smaller, finely pitted and have a thinner eggshell than the eggs of *T. canis*. This observation was in agreement with Schantz and Glickman (1978) and Urquhart *et al*. (1989) who described the eggshell of *T. canis* as thick and pitted or corrugated.

Though both *T. canis* and *T. cati* eggs have surface pittations that are not clearly observed under light microscopy, scanning electron microscopic examinations revealed the differences in the surface pits and ridges of the eggs. Eggs of *T. canis* have coarse, large, deep surface depressions and ridges whereas those of *T. cati* have fine, smaller, and numerous pits. This observation is consistent with the description of Ubelaker and Allison (1975), Uga, Matsuo, Kimura, Rai, Koshino and Igarashi (2000), and Overgaaw and Boersema (1998a and 1998b). According to Ubelaker and Allison (1975), the depressions of the surface pits of the eggs of *T. canis* appear large and craterlike whereas they are smaller and cavelike in *T. cati*.

In the present study, it was found that *T. canis* eggs (83.55 μm by 73.43 μm) were on average slightly larger than those of *T. cati* (73.07 by 56.5 μm). Similar egg-size descriptions were given by Olsen (1974), Schantz and Glickman (1978), Soulsby (1982) and Kassai (1999). However, some *T. cati* eggs were found to overlap with the size ranges of *T. canis* eggs and a similar observation was also reported by Uga *et al*. (1996).

It is therefore, difficult to distinguish between eggs of *T. canis* and *T. cati* based on their size alone.

Unlike the eggs of *T. canis* and *T. cati*, the eggs of *T. leonina* are morphologically distinct using both light and scanning electron microscopy. The eggs are slightly oval in shape, larger in size than those of *Toxocara* spp. and have a smooth surface. These morphological differences make it easy to distinguish *T. leonina* eggs from those of *Toxocara* spp., in mixed infection of domestic pets.

Larval stages

Second-stage larvae of *T. canis* released from embryonated eggs were morphologically similar to the second-stage larvae recovered from the tissues of infected mice. This observation was in agreement with Beaver *et al*. (1952), Nichols (1956) and Sprent (1958) who reported that second-stage larvae recovered from infected mice were indistinguishable from the infective second-stage larvae recovered from the eggs.

The size of *T. canis* second stage larvae recovered from embryonated eggs was somewhat smaller (average: 382.98 by 20.38 μm) than the size of second stage larvae (average: 415.24 by 22.39 μm) recovered from tissues of infected mice. However, there is no difference in the ranges of the maximum size measurement as they are nearly equal. This indicates that there was no growth of the second stage larvae while in the tissues of mice. The reason for the large difference in the minimum size measurements of the larval stages released from eggs and those from the tissues of infected mice was not clear. All

larval stages released from the eggs were considered to be second-stage larvae, however, of these could still have been first-stage larvae when released.

Size measurements of second-stage *T. canis* larvae released from eggs were reported as 404 (360 – 434) μm for larvae expressed from eggs and heat-killed (Nichols 1956), 390 – 440 μm for larvae expressed from eggs and cleared in glycerin (Sprent 1958) and 375 μm x 18 – 22 μm for larvae expressed from eggs and heat killed (Beaver *et al*. 1952). Reported size measurements of second stage larvae from the tissues of mice were 357 - 445 μm (Nichols 1956), 350 μm x 20 μm (Beaver *et al*. 1952) and 630 μm x 30 μm (De Souza and Nakashima 1995). Wang *et al*. (1983) reported a size measurement of 450 μm x 20 μm for second-stage larvae recovered from the tissues of human beings. A size measurement of 340 – 430 μm was reported for second stage larvae from infected dogs cleared in glycerin (Sprent 1958).

In the present study the size measurements of second-stage larvae of *T. cati* released from the eggs were 394.12 μm x 18.82 μm and 389.38 μm x 19.00 μm for larvae from infected tissues of mice. This shows that the larvae did not undergo growth in the mouse tissues. Sprent (1956) reported a size of 312 – 423 μm for larvae expressed from eggs and heat killed, 306 – 458 μm for larvae recovered from mice, and 306 - 459 μm for larvae recovered from tissues of cats. Nichols (1956) also reported the size of *T. cati* second stage larvae as 404 μm x 16 μm for larvae expressed from eggs and 394 – 414 μm x 16 μm for larvae recovered from mouse tissues.

According to Nichols (1956), a wide variation in length may occur in larvae of either *T. canis* or *T. cati* recovered by digestion of tissues from mice or second stage larvae pressed from eggs. He suggested that the difference in size of larvae recovered from mice resulted not from growth in the tissues but from the inherent variation in size of the infective larvae**.** In the present study, morphological differences between second stage larvae of *T. canis* and *T. cati* were not observed. Only slight size variations occurred. According to Nichols (1956), infective-stages of *T. canis* and *T. cati* have an almost identical morphology. They differ little from each other, *T. cati* being slightly smaller and its lateral alae somewhat longer (Nichols 1956).

In the present study it was observed that larvae of *T. canis* and *T. cati* were more vigorously motile than the larvae of *T. leonina*. In spite of this, it was easier to detect the larvae of *T. leonina* compared to the larvae of *T*. canis and *T. cati* recovered from the tissues of infected mice under the microscope. When preserved with a fixative (5% formol saline), the larvae of *T. cati* more often became coiled than the larvae of *T. canis*.

In the present study, size measurements of second-stage larvae of *T. leonina* from eggs were 234.63 μm x 18.79 μm compared to 648.73 x 30.55 μm for those recovered from the tissues of infected mice. These size measurements indicated major differences in the average, minimum and maximum ranges between second-stage larvae released from the eggs and larvae recovered from the tissues of mice. Similar size ranges were reported by Sprent (1959). He reported size ranges of 190 – 220 μm for second-stage larvae pressed from the eggs, 206 – 700 μm for larvae recovered from tissues of mice infected with the

canine strain of *T. leonina* and 185 - 639 μm for larvae recovered from the tissues of mice infected with the feline strain of *T. leonina*. In addition to size differences between larvae from eggs and those from mouse tissues, it has been observed that larvae recovered from the tissues of mice have more distinct external and internal morphological features than those larvae recovered from the eggs. This observation indicates that the larvae of *T. leonina* might have moulted to third-stage larvae in the tissues of infected mice.

Adult worms

Gross morphological examination has shown that adult worms of *T. canis*, *T. cati* and *T. leonina* are similar in general appearance and colour. However, closer observation revealed many differences between the three species. Some of the major differences observed in the morphology of adult worms of the three parasites regarded body curvature, body length, shape of the cervical alae, presence or absence of esophageal ventriculation, position of the vulva in female worms and shape of the tail in male worms.

The anterior body of *T. canis* and *T. cati* curved ventrally, unlike *T. leonina*, which was bent dorsally. This was in agreement with the description given by Soulsby (1982) and Roberts and Janovy (2000). As far as size is concerned, *T. canis* females were the largest worms recovered from domestic pets during necropsy, followed by *T. cati* and *T. leonina* females. The adult female *T. canis* has been described as the largest ascarid occurring in canines, with size measurements ranging from 10-22 cm (Soulsby 1982; O'Lorcain

1994). However, in the present study one adult female of 27.5 cm in length and 0.25 cm in width was recovered. This is the largest adult female ascarid worm ever recovered from a dog. Size measurements of adult male worms showed that *T. canis* was again the largest (9.09 cm) followed by *T. cati* (6.09 cm) and *T. leonina* (6.18 cm).

Toxocara canis and *T. cati* may be differentiated by means of gross examination of the shape of the cervical alae. Those of *T. canis* are spear-shaped, tapering gradually whereas *T. cati* has arrow-shaped cervical alae with the posterior margins almost at right angles to the body (Urquhart *et al*. 1989).

Since both *T. canis* and *T. leonina* infect dogs and both have long and spear-shaped cervical alae (Okoshi and Usui 1967a), it was difficult to distinguish the adult worms. However, light microscopic examination of the tail of the male worms aids with identification as the males of *T. canis* have a finger-like prolongation at the end of the tail which is absent from the gradually tapering tail of *T. leonina*. Furthermore, the presence of a median papilla anterior to the cloaca in males of *Toxocara* spp. and the arrangement of the caudal papillae in males of *Toxocara* spp. and *T. leonina* are specific and can be used for identification of the species. The presence of a median papilla anterior to the cloaca in male *T. canis* and *T. cati* has not been reported before. The present study revealed this structure for the first time in *Toxocara* spp. from domestic pets.

It was difficult to differentiate *T. canis* and *T. leonina* female worms based on the external morphology alone. However, mature female worms were accurately

differentiated on the basis of the morphology of their eggs taken from the uterus. Only mature female worms can be distinguished in this way (O'Lorcain 1994). According to Doganay and Oge (1993), the presence or absence of a ventriculus intercalated between the esophagus and intestine is an important criterion in the identification of immature and adult *T. leonina* and *T. canis* worms. *Toxocara* species have a distinct ventriculus between the esophagus and the intestine, a structure absent in *T. leonina* (Sprent 1959, Sprent and Barrett 1964). Similar observations were reported by Fisher, Murphy and Siedek (2002). According to them, *T. leonina* can be distinguished from *Toxocara canis* primarily by the absence of an esophageal ventriculus. Other features such as the appearance of the eggs in the adult female worm and the shape of the male tail were also used to confirm the identity of the worms.

In conclusion, whereas eggs and adults of the ascarid species *T. canis*, *T. cati* and *T. leonina* can be distinguished without too much difficulty, the developmental stages are morphologically closely similar and almost impossible to distinguish. Factors that can contribute towards the differentiation of the larval stages are the source of the developmental stages (hosts), as well as detailed morphological studies using a combination of light and scanning electron microscopic techniques. This study was conducted on developmental stages of ascarids from experimentally infected domestic pets. Further studies should be directed towards naturally infected domestic pets, wild carnivores and paratenic hosts to determine the extent of possible morphological variation among larvae from these natural sources.

CHAPTER III

TRANSPLACENTAL AND TRANSMAMMARY MODES OF TRANSMISSION OF *TOXOCARA CANIS, TOXOCARA CATI* AND *TOXASCARIS LEONINA* IN A PARATENIC HOST

3.1 *ABSTRACT*

Transplacental infection with Toxocara canis (Werner, 1782) and transmammary infection with Toxocara cati (Schrank, 1788) are the major routes of transmission in dogs and cats, respectively. Rodents, humans and other animals may act as paratenic hosts. The aims of this study were to determine the possible transplacental and transmammary transmission of these parasites as well as Toxascaris leonina (V. Linstow, 1902) in mice and if transmission occurred, to determine whether an infection acquired before or during pregnancy is transmitted. Thirty-four female mice were infected prior to and 18 during pregnancy. The infected mice as well as the fetuses and newborns were dissected before or after giving birth. Tissues and organs were digested using artificial gastric juice and the larvae were recovered with the aid of a Baermann apparatus. Larval burdens were determined. No larvae were found in the fetuses, placentas or uteri of mice infected before pregnancy and dissected before or after giving birth. Larvae of T. canis and T. cati were recovered from newborns and mammary tissues. Larvae of T. canis were recovered from the placenta, uteri, mammary tissues and newborns of mice infected during pregnancy and dissected before or after giving birth. Larvae were recovered in newborns and mammary tissues of mice infected with T. cati, but no larvae were recovered from the placenta, uteri or fetuses. None of the mice infected with T. leonina had larvae in the placenta, uteri, mammary tissues, fetuses or newborns. This study

indicated the possible transplacental transmission in mice infected with T. canis during pregnancy, and possible transmammary transmission when infected prior to or during pregnancy. Although possible transmammary transmission of T. cati from infections acquired prior to or during pregnancy was observed, there was no evidence for transplacental transmission. Neither transplacental nor transmammary transmission was observed in mice infected with T. leonina.

Keywords: *Toxocara canis, Toxocara cati, Toxascaris leonina, transplacental transmission, transmammary transmission, mice, paratenic host.*

3.2 INTRODUCTION

Dogs and other canids are the definitive hosts of *Toxocara canis*, while cats and other felines are the definitive hosts of *Toxocara cati* (Dubinsky, Havasiova-reiterova, Petko, Hovorka and Tomasovicova 1995). Both dogs and cats are the definitive hosts of *Toxascaris leonina*.

Potential paratenic hosts for *T. canis* and *T. cati* include earthworms, cockroaches, mice, rats, chickens, birds, pigs and lambs (Sprent 1956 and 1958). Humans are also considered as paratenic hosts for *T. canis* and *T. cati* (Glickman and Schantz 1981) while mice, chickens and earthworms can act as paratenic hosts for *T. leonina* (Sprent 1959; Okoshi and Usui 1968).

Toxocara canis in dogs and *T. cati* in cats have complex life cycles. Dogs acquire infections of *T. canis* through ingestion of infective eggs, eating infected meat of paratenic hosts, ingestion of larvae in breast milk (transmammary transmission) and/or transplacental migration of larvae in pregnant bitches to the fetuses across the placental barrier (Glickman and Schantz 1981; Kassai 1999). The transplacental route is the major route of transmission of *T. canis,* ensuring that almost all puppies from infected bitches are born infected (Wang *et al*. 1983; Taylor, O'Connor, Hinson and Smith 1996; Overgaauw 1997b). The larvae hatch in young dogs (< 6 months old), penetrate the intestinal wall and follow the heart-lung-tracheal migration route to finally develop into adult worms in the intestine. The larvae follow a somatic migration in older dogs (> 6 months old), and do not develop into adult worms (Glickman and Schantz 1981),

however, reactivated somatic larvae or larvae from a newly acquired infection may undergo tracheal migration and develop into adult worms in pregnant and lactating bitches. Reactivated somatic larvae also migrate across the placenta and through the breast milk to infect fetuses and newborn puppies. The larvae from fetal infections become sexually mature in the intestine of puppies at the age of two to three weeks (Soulsby 1982). Infections from paratenic hosts such as mice do not result in the somatic migration of the larvae, but the larvae develop directly into adult worms in the intestine (Kassai 1999).

Cats get infected with *T. cati* by ingesting infective eggs, eating infected meat of paratenic hosts and/or ingesting larvae through breast milk (Kassai 1999). However, unlike *T. canis* in dogs, there is no transplacental route of transmission of *T. cati* in cats (Sprent 1956; Jacobs, Arakawa, Courtney, Gemmell, McCall, Myers and Vanparijs 1994). Transmammary migration of larvae to kittens (Kassai 1999) and eating infected meat of paratenic hosts are the major routes of transmission in cats (Dubinsky *et al*. 1995). Age resistance to *T. cati* was not reported in cats and the larvae hatch, penetrate the intestinal wall and follow the heart-lung-tracheal migration route to finally develop into adult worms in the intestine.

Dogs and cats are infected with *T. leonina* through the ingestion of infective eggs and eating the meat of paratenic hosts containing the larval stages. No migrations of larvae occur but the larvae develop into adult worms in the intestine (Soulsby 1982; Kassai 1999). *Toxascaris leonina* patent infections are more commonly found in adult dogs and

cats (Parsons 1987; Jacobs *et al*. 1994) and paratenic hosts are considered to be the major source of infections (Soulsby 1982; Dubinsky *et al*. 1995). Transplacental or transmammary transmission to puppies or kittens does not occur in *T. leonina* (Jacobs *et al*. 1994).

The larvae of *T. canis* and *T. cati* have no heart-lung and tracheal migration to develop into adult worms in paratenic hosts, such as mice, humans and other animals, but undergo somatic migration and remain as larvae in tissues and organs (Glickman and Schantz 1981). The larvae migrated to the somatic tissues such as the liver, lungs and muscles, but did not develop into adult worms in sheep (Aldawek, Levkut, Revajova, Kolodzieyski, Seveikova and Dubinsky 2002), rabbits (Dzbenski, Bitkowska and Golab 1999) and mice (Sprent 1956 and 1958) experimentally infected with infective eggs of *Toxocara* spp. Small mammals play a significant role as a reservoir of infections in the circulation and maintenance of ascarids (Soulsby 1982; Dubinsky *et al*. 1995), and mice can be used as models to study modes of transmissions, and the distribution of larval *Toxocara* infections (Lee, Min and Soh 1976; Hassan and El-Manawaty 1994; Holland and Cox 2001).

Although the general objective of this study was to demonstrate the possible transplacental and transmammary modes of transmission of *T. canis* in NIH inbred mice as model, it also aimed to determine whether transplacental and transmammary transmission occurred as a result of the reactivation of larvae from tissues acquired prior to pregnancy or from infections acquired during pregnancy. An additional objective of

this study was to determine possible transplacental and transmammary modes of transmission in *T. cati* and *T. leonina,* and assess the distribution of the larval stages of the three parasites in the different tissues and organs of infected adult female mice.

This kind of investigation provides opportunity to acquire knowledge on the mode of transmission and the life cycle of these parasites. Besides this, an increase in awareness of the modes of transmissions and life cycles of these parasites may provide better understanding with regard to prevention of infection and control aspects of these parasites.

3.3 MATERIAL AND METHODS

3.3.1 Study Areas and Period

Experimental studies on the transplacental and transmammary mode of transmission of *Toxocara* spp. in laboratory mice was conducted from February to May 2002, in the experimental animal unit, Faculty of Medicine and in the department of Zoology and Entomology, Faculty of Natural Sciences, University of the Free State, Bloemfontein, South Africa.

3.3.2 The Parasites

Ascarid roundworms of dogs and/or cats, i.e. *T. canis*, *T. cati* and *T. leonina*, were used to study the transplacental and the transmammary modes of transmission in a paratenic host (mice). Eggs of *T. canis, T. cati* and *T. leonina* were collected from the uteri of gravid, adult female worms at ClinVet International (Pty) Ltd, Bloemfontein, South Africa. The eggs were stored in 0.05 mol H_2SO_4 solution as a culture medium (Prof. D.J. Kok, 2001, personal communication) in the dark at room temperature and were checked weekly for embryonation.

3.2.3 The Research Animals

3.2.3.1 Type

National Institute of Health (NIH) inbred, female, non-pregnant mice were obtained from the stock of the Experimental Animal Unit, the Medical Faculty of the University of the Free State.

3.3.3.2 Maintenance

The female mice were housed in cages measuring 35cm x 15cm x 13cm and maintained in the Experimental Animal Unit (Fig. 3.1). The mice were maintained at constant environmental conditions, temperature at 21^0C $\pm$ 1^0C, humidity of 50% $\pm$ 10% RH and day/night cycle at 12 hours light and 12 hours dark. The cages were kept clean; saw dust, renewed weekly, was used as bedding and nesting. The mice were kept in the same room after infection with *T. canis, T. cati* or *T. leonina,* but in separate cages to avoid possible contamination of the environment with the faeces of infected mice and to avoid possible cross infections. The mice were fed commercial rodent food and they had free access to food and water. The mice were acclimatized to the experimental environment for at least seven days before exposure to the infective stages of the parasites.

Fig 3.1. Mice maintained in cages at the Experimental Animal Unit, Free State University.

3.3.4 Ethical considerations

Consent was obtained from the Ethical Committee of the University of the Free State to use mice as an experimental animal model for the demonstration of transplacental and/or transmammary transmission of larval stages of *T. canis, T. cati* and *T. leonina.* The adult female mice were killed by cervical dislocation and the newborns were decapitated. This

was done according to accepted standard procedures for the humane killing of laboratory animals for experimental purposes.

3.3.5 Experimental design

The experimental study was divided into two phases. Phase I, as shown in Fig. 3.2, was designed to determine the possible transplacental and the transmammary modes of transmission from mother to fetus from infections acquired prior to pregnancy, whereas phase II was designed to determine the possible transplacental and the transmammary modes of transmission from the mother to the fetus from infections acquired during pregnancy (Fig. 3.3). Two subgroups of mice, I and II, were used in the two consecutive phases of the study.

Although it was planned to infect equal numbers of mice with *T. canis*, *T. cati*, or *T. leonina*, equal numbers of mice could not be infected due to the number of eggs available at the time of experimental infection. A total number of 52 female mice were used, i.e. 34 and 18 in subgroups I and II, respectively. In subgroup I, 14 mice were infected with *T. canis*, 14 with *T. cati* and six with *T. leonina*. In subgroup II, 10 mice were infected with *T. canis*, six with *T. cati* and two with *T. leonina*. Five *T. canis*, two *T. cati* and one *T. leonina* infected mice died before results could be obtained from them in study subgroup I, while two *T. canis* and one *T. cati* infected mice died in subgroup II. The mice that died were not considered as part of the “fit for analysis” study populations.

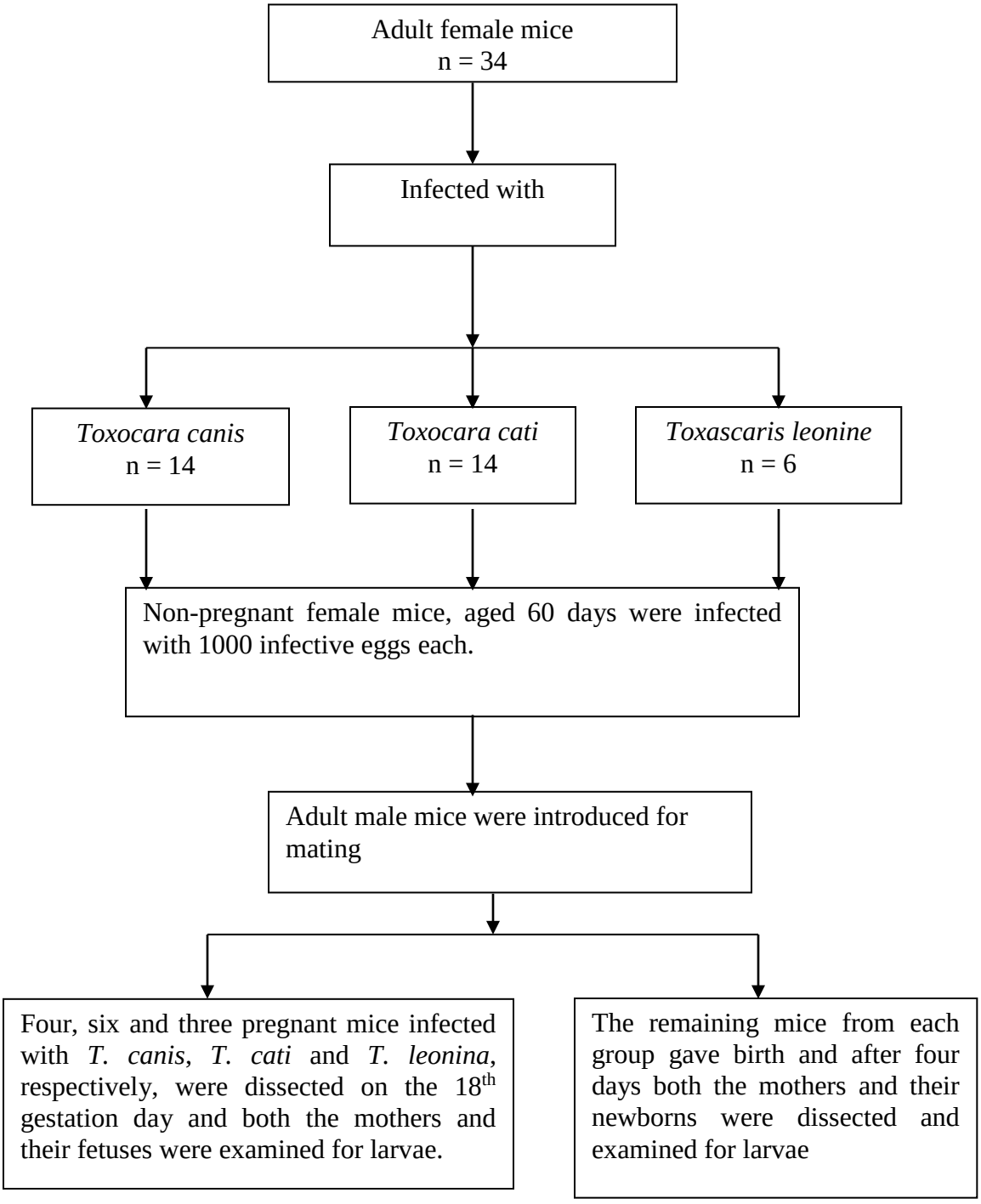


Figure. 3.2. Study subgroup I. Chart showing the steps taken in the study of the transplacental and the transmammary modes of transmission of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) in mice from infections acquired prior to pregnancy.

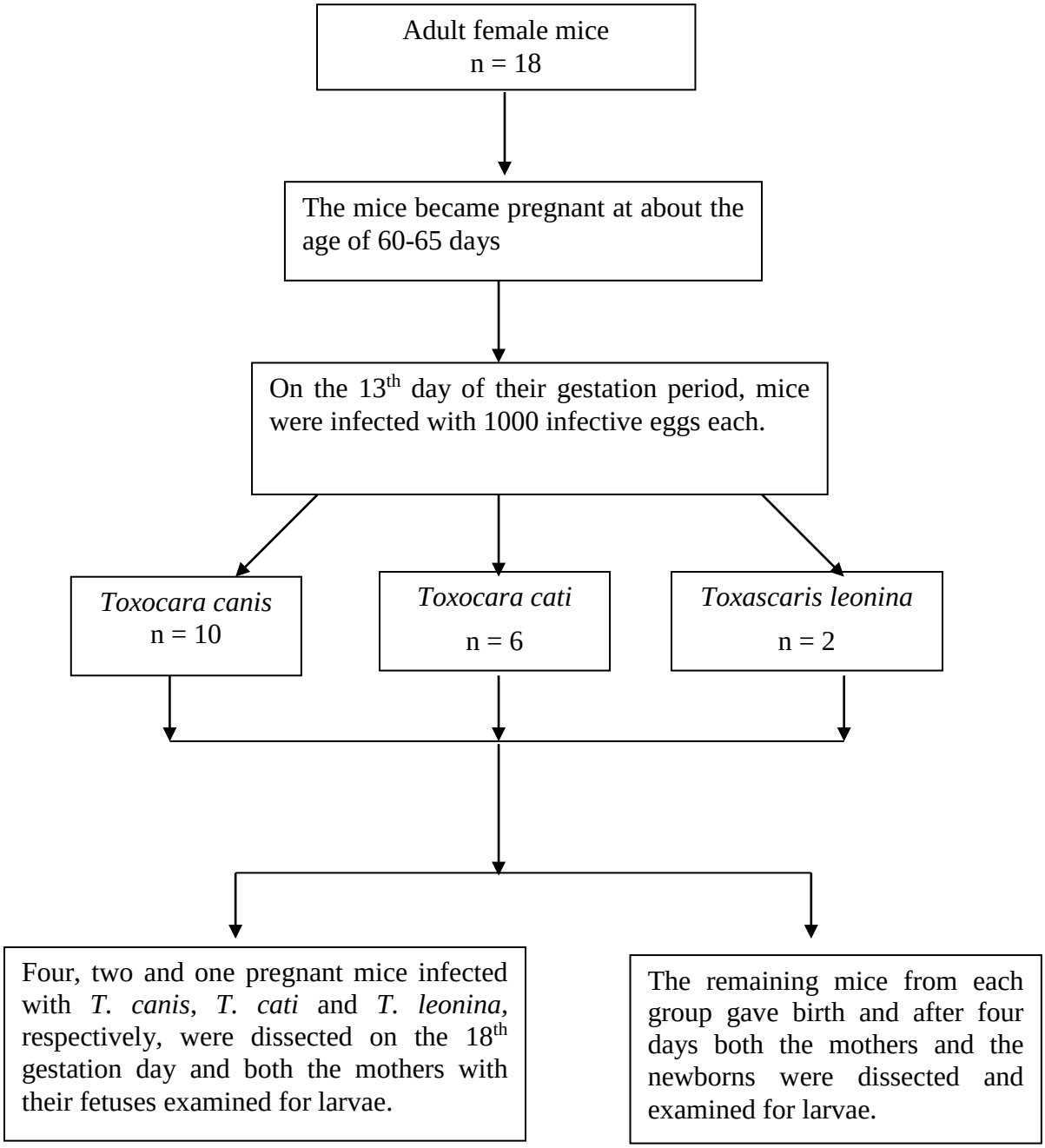


Figure 3.3. Study subgroup II. Chart showing steps taken in the study of the transplacental and the transmammary modes of transmission of *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) in mice from infections acquired during pregnancy.

3.3.5.1 Infecting Mice

Mice were each infected with 1000 eggs of one of the parasite species, *T. canis, T. cati* or *T. leonina*. Fully embryonated eggs were washed repeatedly with distilled water and counted under a stereomicroscope. Counted eggs were suspended in 1 ml distilled water and the mice were infected orally, once only, using a 1 ml syringe fitted with a specially designed round-tipped needle. Female mice were infected at the age of 60 days for the study to assess transplacental and transmammary transmission a pre-pregnancy acquired infection. A male mouse was introduced to each infected female ten days later. The first day of gestation was determined by examination for the presence of a vaginal plug (Oshima 1961a). In the study to determine transplacental and transmammary transmission from an infection acquired during pregnancy, the female mice at the age of 60 days were individually caged with a male for mating. The female mice were infected on the 13^{th} day after pregnancy was established.

3.3.5.2. Recovery of Larvae

The larvae of the parasites from the mothers, fetuses and newborns were recovered using an artificial gastric juice digestion method. Fetuses or newborns of a mouse were digested together as a pool. As the gestation period in mice is at least 19 days, about half of the pregnant female mice were dissected on the 18^{th} day before giving birth, to investigate the transplacental route of transmission in the fetuses for each parasite species (Fig. 3.4). For the investigation of the transmammary transmission of the parasite from the mother to the newborn through the breast milk, the newborn mice were allowed to stay with their mothers allowing them to breast feed for four days before they were dissected. The

artificial digestive fluid used was 1% pepsin in a hydrochloric acid solution (pH1) (5g pepsin, 7ml HCl, and 1000 ml distilled water; Garcia and Ash 1979). The liver, lung, heart, kidney, diaphragm, mammary tissues, placenta, uterus, gastrointestinal tissues (GIT), carcass (skeleton and muscular tissues) of the mother, whole fetuses and whole newborns were separately minced, weighed and placed in an Erlenmeyer flask. The digestive fluid was used in the ratio of 1 part tissue to 20 parts fluid and the content was incubated overnight at 37^{o}C. The brain was sectioned into small pieces, pressed between two slides and microscopically examined for larval stages (Kayes and Oaks 1976; Garcia and Ash 1979).

The Baermann procedure (Garcia and Ash 1979; Kassai 1999) was carried out to detect, identify and count the total number of larvae from each specimen during stereomicroscopic examination of digested tissues. A 20 cm glass funnel closed at the bottom with a clamped piece of rubber tubing, a Pasteur pipette attached to its end, was fixed to a stand. A circular tea filter was placed in the funnel and on it a circular disc of nylon gauze. Warm water (37^{0} C) was added to the digested tissue mixture to triple its original volume and poured into the funnel. The setup was left for 1-2 hours for the larvae to collect at the bottom, before releasing the pinch clamp to release 2-3 ml of fluid into a petridish for examination. All the digested tissue samples were examined by the same procedure. In the case of small volumes (e.g. kidney, spleen, heart, diaphragm) the whole sample diluted with warm water and examined directly without following the Baermann procedure. Particular attention was given to the placenta, uterus and mammary tissues of adult female mice.

Figure 3.4. A mouse infected with *Toxocara canis* (Werner, 1782) and dissected before giving birth. The 12 live fetuses and two dead fetuses in the uterus (arrow) can be seen.

3.3.5.3. Statistical Analysis

Descriptive statistics were used to analyze the data. The percentage recovery rate (PRR) of larvae from a mouse expressed in terms of the 1000 infective eggs administered to each mouse, the average percentage recovery rate (APRR) of larvae in mice infected prior to or during pregnancy and dissected before or after giving birth (Lee *et al*. 1976), mean intensity (MI) and relative density (RD) (Kassai 1999) of infections were calculated using the following formulas:

$$\text{PRR} = \frac{\text{Total number of larvae recovered from a mouse and from its progeny}}{1000\ (eggs)} \times 100$$

$$\text{APRR} = \frac{\text{Average number of larvae recovered from from several mice}}{1000\ (eggs)} \times 100$$

$$\text{MI} = \frac{\text{Total number of parasites of a particular species}}{\text{Infected host individuals in the sample}} \times 100$$

$$\text{Relative density} = \frac{\text{Total number of parasites of a particular species}}{\text{Infected + uninfected host individuals in the sample}} \times 100$$

3.4 RESULTS

3.4.1 Transmission

The number of larvae recovered from *T. canis, T. cati or T. leonina* infected mice prior to or during pregnancy and from their progeny (fetuses and newborns) is summarized in Table 3.1.

***Toxocara canis* (Werner, 1782)**

Four of the nine adult female mice infected with *T. canis* prior to pregnancy, were dissected before giving birth. All were positive and 320 larval stages were recovered from their tissues and organs. No larval stages were recovered from their 41 fetuses. Four of the remaining five adult female mice that were dissected post partum, were positive and a total of 277 larval stages recovered. Three larvae were collectively recovered from their 45 newborns.

Four of the eight pregnant mice infected with *T. canis* during pregnancy, were dissected before giving birth. All were positive and 332 larval stages were found in their tissues. Fifteen larval stages were collectively recovered from the 49 fetuses. The four female mice that were dissected post partum all were positive and 424 larvae were recovered from their tissues. Seventeen larvae were collectively recovered from their 47 newborns.

***Toxocara cati* (Schrank, 1788)**

A total of twelve female mice were infected with *T. cati* prior to pregnancy. Six of them were dissected before giving birth. Of these, five were positive and 152 larvae were

recovered. No larval stages were recovered from the 70 fetuses. From the remaining six mice, dissected post partum, five were positive and 170 larvae were found in their tissues and organs. Only four larvae were recovered from their 41 newborns.

Five female mice were infected with *T. cati* during pregnancy. Of these, two were dissected before giving birth. Both were infected and 149 larvae were recovered. No larval stages were recovered from the 27 fetuses. The remaining three mice were dissected post partum. All three were positive and 219 larval stages were recovered. Eighteen larval stages were recovered from the twenty-eight newborns.

Toxascaris leonina (V. Linstow, 1902)

Three of the five female mice infected with *T. leonina* prior to pregnancy were dissected before giving birth and two were positive. Sixty-seven larvae were recovered from them. None of the 40 fetuses were infected. The remaining two females were dissected post partum and one was positive. Forty-one larval stages were recovered. No larval stages were found in the 16 newborns.

Two female mice were infected with *T. leonina* during pregnancy. One mouse was dissected before giving birth and 70 larval stages were recovered from it. No larval stages were found in its 13 fetuses. Forty-one larvae were recovered in the mouse dissected post partum but none was found in the 14 newborns.

Table 3.1. Number of larvae recovered from *Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (V. Linstow, 1902) mice infected prior to or during pregnancy and dissected before or after giving birth, their fetuses and newborns.

		Infected prior to pregnancy				Infected during pregnancy			
Parasites	Source	DBGB	NLR	DAGB	NLR	DBGB	NLR	DAGB	NLR
T. canis	Adults	4	320	5	277	4	332	4	424
	Fetuses	41	0	-	-	49	15	-	-
	Newborns	-	-	45	3	-	-	47	17
T. cati	Adults	6	152	6	170	2	149	3	219
	Fetuses	70	0	-	-	27	0	-	-
	Newborns	-	-	41	4	-	-	28	18
T. leonina	Adults	3	67	2	41	1	70	1	41
	Fetuses	40	0	-	-	13	0	-	-
	Newborns	-	-	16	0	-	-	14	0

DAGB = Dissected After Giving Birth

DBGB = Dissected Before Giving Birth

NLR = Number of Larvae Recovered

3.4.2 Distribution and Recovery Rate of Larvae

In the study of the possible transplacental and transmammary modes of transmission of *T. canis, T. cati* and *T. leonina* in mice, the distribution and larval burdens in tissues and organs of adult female mice infected prior to or during pregnancy and dissected before or after giving birth were also determined and these results are shown in Table 3.2 to Table 3.7.

***Toxocara canis* (Werner, 1782)**

A mean intensity of 80.0 larvae per mouse was recovered in four mice that were infected with *T. canis* prior to pregnancy and dissected before giving birth. The highest numbers of larvae recovered were in the carcass (45%), followed by the brain (39.1%), mammary tissues (7.5%). The lowest numbers were from the diaphragm (0.6%) and kidney (0.6%). Four of the five mice infected prior to pregnancy and dissected post partum were positive, and a mean intensity of 70.0 larvae were found per mouse. Like those dissected before giving birth, many larvae were recovered from the carcass (43.9%), brain (40.7%) and mammary tissues (5.7%), and few were recovered from the liver (0.4%). But in this case 1.1% of larvae were recovered from the newborns. Although larvae were recovered in the mammary tissues of mice dissected before or after giving birth and in the newborns, larvae were not found in the placenta, uterus or fetuses. The average percentage recovery rate of larvae from mice infected prior to pregnancy and dissected before giving birth was 8.0% (range 6.5 % to 10.5 %). The average percentage recovery rate of larvae from mice infected prior to pregnancy and dissected post partum was 7.0% (range 5.9 % to 7.8 %) (Table 3.2).

Table 3.2. Distribution and percentage recovery rate of *Toxocara canis* (Werner, 1782) larvae in the tissues and organs of mice infected prior to pregnancy and dissected before (n = 4) or after (n= 5 of which 4 were positive) giving birth.

	Dissected before giving birth						Dissected after giving birth					
Source	A1	A2	A3	A4	Total (%)	Mean	B1	B2	B3	B4	Total (%)	Mean
Brain	38	28	27	32	125 (39.1)	31.3	29	23	38	24	114 (40.7)	28.5
Liver	2	1	0	1	4 (1.3)	1.0	0	0	1	0	1 (0.4)	0.3
Lung	2	3	0	2	7 (2.2)	1.8	3	1	3	2	9 (3.2)	2.3
Uterus	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	0	0	0	0	0	0	Not examined					
Mammary tissues	9	3	7	5	24 (7.5)	6.0	4	5	3	4	16 (5.7)	4.0
Heart	3	0	1	1	5 (1.6)	1.3	0	1	1	0	2 (0.7)	0.5
Diaphragm	0	1	0	1	2 (0.6)	0.5	0	2	0	3	5 (1.8)	1.3
Kidney	0	2	0	0	2 (0.6)	0.5	0	1	2	1	4 (1.4)	1.0
Carcass	48	27	43	26	144 (45.0)	36.0	32	38	30	23	123 (43.9)	30.8
GI tissues	3	0	2	2	7 (2.2)	1.8	1	0	0	2	3 (1.1)	0.8
Fetuses	0	0	0	0	0	0	Not applicable					
Newborns	Not applicable						1	2	0	0	3 (1.1)	0.8
Total	105	65	80	70	320 (100)	80.0	69	71	78	59	280 (100)	70.0
Recovery rate (%)	10.5	6.5	8.0	7.0		8.0*	6.9	7.1	7.8	5.9		7.0*

*Average percentage recovery rate A1-A4 = Mice dissected before giving birth

B1-B4 = Mice dissected after giving birth GI = Gastrointestinal tissues

A mean intensity of 86.8 larvae were recovered from the four adult female mice infected with *T. canis* during pregnancy and dissected before giving birth. Most of the larvae were again from the carcass (35.5%), brain (15.6%) and mammary tissues (12.4%). In these mice some larvae were also recovered from the uterus (1.7%), placenta (2.8%) and fetuses (4.3%). From the four adult female mice infected during pregnancy and dissected after giving birth, the mean intensity of larvae was 110.3. Most of the larvae were found in the carcass (42.18%), mammary tissues (20.6%) and brain (14.1%). Larvae were also collected from the uterus (1.8%) and newborns (3.85%). The average percentage recovery rate of *T. canis* larvae from mice infected during pregnancy and dissected before giving birth was 8.7% (range 7.9% to 9.8%). The average percentage recovery rate of larvae from mice infected during pregnancy and dissected post partum was 11.0% (range 9.3% to 12.2 %) (Table 3.3).

Table 3.3. Distribution and percentage recovery rate of *Toxocara canis* (Werner, 1782) larvae in the tissues and organs of individual mice infected during pregnancy and dissected before (n = 4) or after (n = 4) giving birth.

	Dissected before giving birth						Dissected after giving birth					
Source	A1	A2	A3	A4	Total (%)	Mean	B1	B2	B3	B4	Total (%)	Mean
Brain	19	13	8	14	54 (15.6)	13.5	28	10	15	9	62 (14.1)	15.5
Liver	5	3	7	0	15 (4.3)	3.8	3	0	1	2	6 (1.4)	1.5
Lung	3	11	7	9	30 (8.6)	7.5	8	5	6	7	26 (5.9)	6.5
Uterus	2	0	1	3	6 (1.7)	1.5	1	4	0	3	8 (1.8)	2.0
Placenta	2	2	3	3	10 (2.9)	2.5	Not examined					
Mammary tissues	15	12	10	6	43 (12.4)	10.8	14	33	17	27	91 (20.6)	22.8
Heart	3	7	0	1	11 (3.2)	2.8	6	3	4	2	15 (3.4)	3.8
Diaphragm	0	3	5	1	9 (2.6)	2.3	2	0	3	3	8 (1.8)	2.0
Kidney	3	6	4	2	15 (4.3)	3.8	3	1	5	2	11 (2.5)	2.8
Carcass	25	29	32	37	123 (35.4)	30.8	51	49	55	31	186 (42.2)	46.5
GI tissues	5	8	3	0	16 (4.6)	4.0	4	2	0	5	11 (2.5)	2.8
Fetuses	3	4	5	3	15 (4.3)	3.8	Not applicable					
Newborns	Not applicable						2	6	5	4	17 (3.9)	4.3
Total	85	98	85	79	347 (100)	86.8	122	113	111	95	441 (100)	110.3
Recovery rate (%)	8.5	9.8	8.5	7.9		8.7*	12.2	11.3	11.1	9.5		11.0*

*Average percentage recovery rate A1-A4 = Mice dissected before giving birth

B1-B4 = Mice dissected after giving birth GI = Gastrointestinal tissues

The mean distribution (%) of *T. canis* larvae in tissues, organs, fetuses and newborns of mice infected prior to (n=9 of which 8 were positive) or during (n=8) pregnancy has been summarized as shown in Fig. 3.5. Larval stages were commonly observed in the carcasses and in the brain.

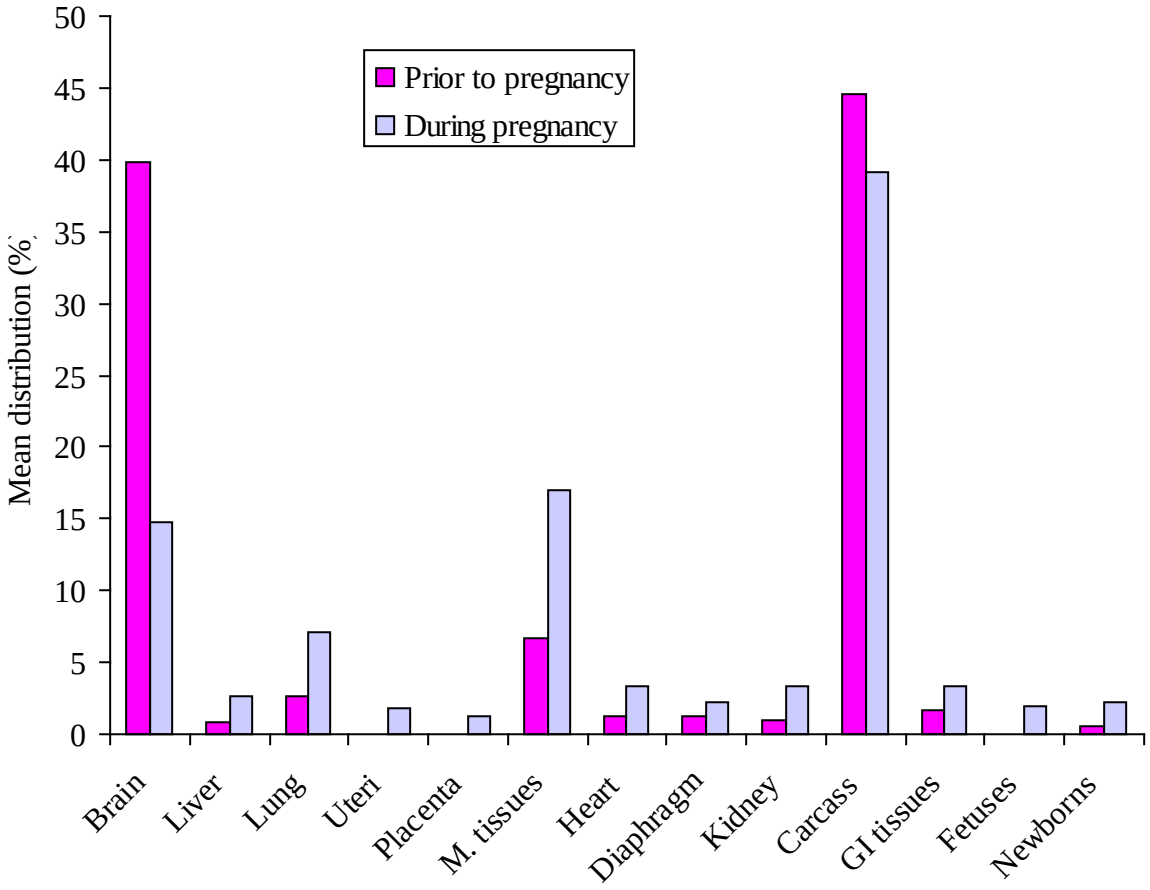


Fig. 3.5. Mean distribution (%) of *Toxocara canis* (Werner, 1782) larvae in tissues organs, fetuses and newborns of mice infected prior to or during pregnancy. GI = Gastrointestinal tissues, M. tissues = Mammary tissues.

***Toxocara cati* (Schrank, 1788)**

From a total of six adult female mice infected prior to pregnancy and dissected before giving birth, 152 *T. cati* larvae with a mean intensity of 25.3 were recovered. The highest numbers of larvae were recovered in the carcass (71.1%), followed by the mammary tissues (11.8%), liver (6.6%), lung (5.26), and lowest numbers were recovered from the heart (0.7%). Larvae were not found in the placenta, uterus, fetuses or brain. From the five positive mice infected prior to pregnancy and dissected post partum, 174 larvae of *T. cati* with a mean intensity of 34.8 and a relative density of 29.0 were recovered. Most larvae were recovered from the carcass (71.8%), mammary tissues (13.5%) and the least were recovered from the kidney (0.6%) and the heart (0.6%). Larvae were not recovered from the uterus. The average percentage recovery rate of *T. cati* larvae from mice infected prior to pregnancy and dissected before giving birth was 2.5% (range 0.7% to 4.7%). The average percentage recovery rate of *T. cati* larvae from mice infected during pregnancy and dissected post partum was 3.5% (range 1% to 4.9 %) (Table 3.4).

Table 3.4. Distribution and percentage recovery rate of *Toxocara cati* (Schrank, 1788) larvae in the tissues and organs of mice infected prior to pregnancy and dissected before (n= 6) or after (n= 6 of which 5 positive) giving birth.

	Dissected before giving birth									Dissected after giving birth							
Source	A1	A2	A3	A4	A5	A6	Total	%	Mean	B1	B2	B3	B4	B5	Total	%	Mean
Brain	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0
Liver	3	1	0	2	2	2	10	6.6	1.7	0	1	3	0	2	6	3.5	1.2
Lung	3	0	1	2	1	1	8	5.3	1.3	0	0	3	5	3	11	6.5	2.2
Uterus	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0
Placenta	0	0	0	0	0	0	0	0.0	0.0	Not examined							
M. Tissues	0	1	0	3	5	9	18	11.8	3.0	0	0	12	3	8	23	13.5	4.6
Heart	0	0	0	0	0	1	1	0.7	0.2	0	0	0	0	1	1	0.6	0.2
Diaphragm	0	0	0	2	1	0	3	2.0	0.5	0	0	0	1	2	3	1.8	0.6
Kidney	0	0	0	0	0	2	2	1.3	0.3	0	0	0	1	0	1	0.6	0.2
Carcass	8	5	7	27	36	25	108	71.1	18.0	10	15	41	26	30	122	71.8	24.4
GI tissues	0	0	0	0	2	0	2	1.3	0.3	0	0	0	2	1	3	1.8	0.6
Fetuses	0	0	0	0	0	0	0	0.0	0.0	Not applicable							
Newborns	Not applicable									0	0	0	2	2	4	2.4	0.8
Total	14	7	8	36	47	40	152	100.0	25.3	10	16	59	40	49	174	102.4	34.8
R. rate (%)	1.4	0.7	0.8	3.6	4.7	4.0			2.5*	1.0	1.6	5.9	4.0	4.9			3.5*

*Average percentage recovery rate A1-A6 = Mice dissected before giving birth,

B1-B5 = Mice dissected after giving birth GI = Gastrointestinal tissues

M. tissues = Mammary tissues R. rate = Recovery rate.

In two mice infected with *T. cati* during pregnancy and dissected before giving birth, 149 larvae with a mean intensity of 74.5 were recovered. Most of the larvae were from the carcass (53.7%), mammary tissues (17.45%), and the least from the heart (1.34%). There were no larvae in the uterus, placenta, fetus or brain. From three mice dissected post partum, 219 larvae with a mean intensity of 79.0 were recovered. Larvae were recovered from the carcass (40.1%), mammary tissues (33.3%) and newborns (7.6%). The lowest numbers of larvae were recovered from the heart (1.3%). Larvae were not recovered from the uterus. The average percentage recovery rate of *T. cati* larvae from mice infected during pregnancy and dissected before giving birth was 7.5% (range 7.0% to 7.9%). The average percentage recovery rate was 7.9% (range 6.4% to 9.0 %) from mice dissected post partum (Table 3.5).

Table 3.5. Distribution and percent recovery rate of *Toxocara cati* (Schrank, 1988) larvae in the tissues and organs of mice infected during pregnancy and dissected before (n=2) or after (n=3) giving birth.

	Dissected before giving birth				Dissected after giving birth				
Source	A1	A2	Total (%)	Mean	B1	B2	B3	Total (%)	Mean
Brain	0	0	0	0	0	0	0	0	0
Liver	1	7	8 (5.4)	4.0	4	2	2	8 (3.4)	2.7
Lung	5	8	13 (8.7)	6.5	5	3	4	12 (5.1)	4.0
Uterus	0	0	0	0	0	0	0	0	0
Placenta	0	0	0	0	Not examined				
Mammary tissues	20	6	26 (17.4)	13.0	20	19	40	79 (33.3)	26.3
Heart	0	2	2 (1.3)	1.0	0	1	2	3 (1.3)	1.0
Diaphragm	0	5	5 (3.4)	2.5	0	2	2	4 (1.7)	1.3
Kidney	3	0	3 (2.0)	1.5	3	1	0	4 (1.7)	1.3
Carcass	45	35	80 (53.7)	40.0	45	27	23	95 (40.1)	31.7
GI tissues	5	7	12 (8.1)	6.0	3	6	5	14 (5.9)	4.7
Fetuses	0	0	0	0	Not applicable				
Newborns	Not applicable				10	3	5	18 (7.6)	6.0
Total	79	70	149 (100)	74.5	90	64	83	237 (100)	79.0
Recovery rate (%)	7.9	7.0		7.5*	9.0	6.4	8.3		7.9*

*Average percentage recovery rate A1-A2 = Mice dissected before giving birth

B1-B3 = Mice dissected after giving birth GI = Gastrointestinal tissues.

The mean distribution (%) of *T. cati* larvae in tissues, organs and newborns of mice infected prior to (n=12 of which 11 were positive) or during (n=5) pregnancy has been summarized as shown in Fig. 3.6. Larval stages were commonly observed in the carcasses and mammary tissues.

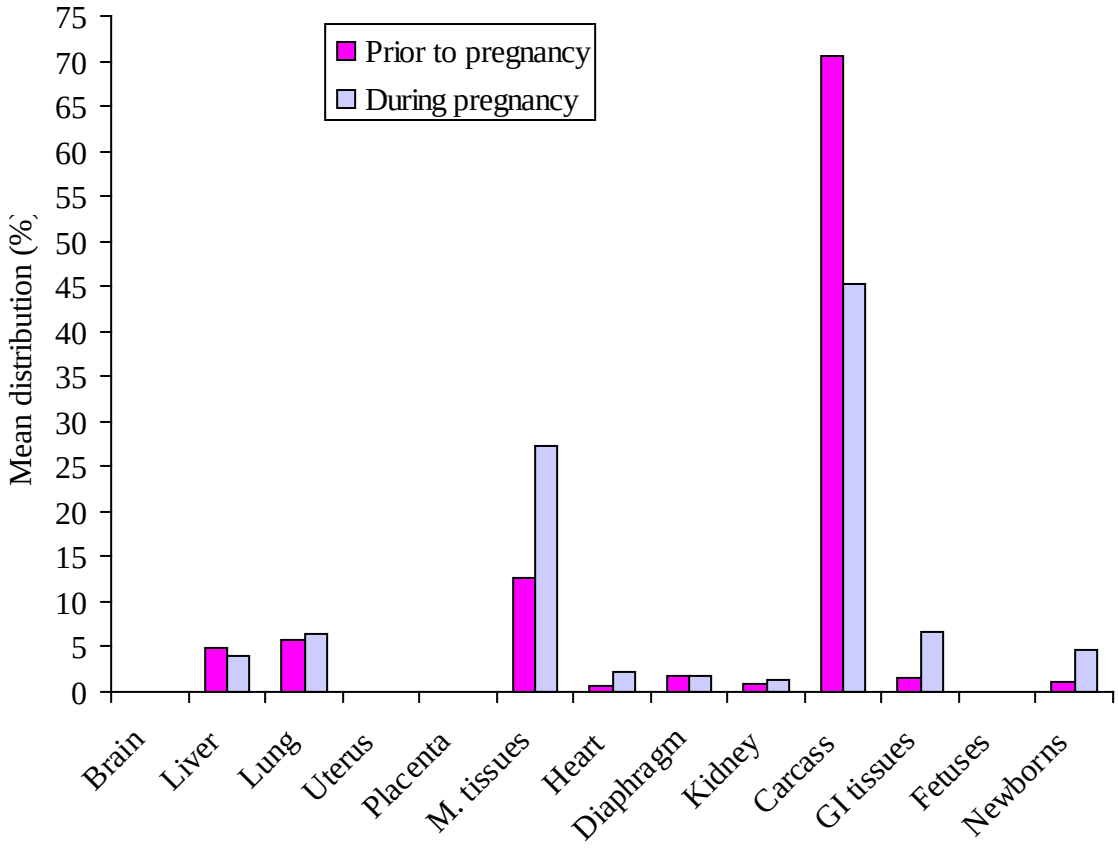


Fig. 3.6. Mean distribution (%) of *Toxocara cati* (Schrank, 1788) larvae in tissues, organs and newborns of mice infected prior to or during pregnancy. GI = Gastrointestinal tissues, M. tissues = Mammary tissues

***Toxascaris leonina* (V. Linstow, 1902)**

Sixty-seven larvae with a mean intensity of 33.5 and a relative density of 22.33 were recovered from mice infected with *T. leonina* prior to pregnancy and dissected before giving birth. Larvae were recovered from the liver (2.99%), lung (35.82%) and carcass (61.19%). Larvae were not recovered from the brain, uterus, placenta, mammary tissues, heart, diaphragm, kidney, gastrointestinal tissues, fetuses and newborns. Only one of the two mice infected with *T. leonina* prior to pregnancy and dissected after giving birth, was infected and 41 larvae were recovered with a relative density of 20.5. Larvae were recovered in the carcass (53.7%), lung (24.4%), gastrointestinal tissue (12.2%) and liver (9.8%). Larvae were not recovered from the brain, uterus, placenta, mammary tissues, heart, diaphragm, kidney, fetuses or newborns. The average percentage recovery rate of *T. leonina* larvae from mice infected during pregnancy and dissected before giving birth was 1.7% (range 2.6% to 4.1%). The percentage recovery rate from mice dissected post partum was 4.1% (Table 3.6). Only two adult female mice were infected during pregnancy and one was dissected before giving birth. Seventy larvae were recovered from the carcass (54.3%), lungs (17.1%), liver (21%), gastrointestinal tissue (4.3%), heart (1.4%) and diaphragm (1.4%). Forty-one larvae were recovered from the carcass (43.9%), lungs (24.4%), gastrointestinal tissue (17.1%), liver (12.2%) and kidneys (2.4%) of the single mouse dissected post partum. Larvae were not recovered from the brain, placenta, uterus, mammary tissues, fetuses and newborns. The percentage recovery rates of *T. leonina* larvae from mice infected during pregnancy and dissected before and after giving birth were 7.0% and 4.1% respectively, (Table 3.7).

Table 3.6. Distribution of *Toxascaris leonina* (V. Linstow 1902) larvae in the tissues and organs of mice infected prior to pregnancy and dissected before (n=3 of which 2 were positive) or after (n=2 of which one was positive) giving birth.

	Dissected before giving birth				Dissected after giving birth	
Source	A1	A2	Total (%)	Mean	B1	% Total
Brain	0	0	0	0	0	0
Liver	0	2	2 (3.0)	1.00	4	9.8
Lung	8	16	24 (35.8)	12.00	10	24.4
Uterus	0	0	0	0	0	0
Placenta	0	0	0	0	Not examined	
M. tissues	0	0	0	0	0	0
Heart	0	0	0	0	0	0
Diaphragm	0	0	0	0	0	0
Kidney	0	0	0	0	0	0
Carcass	33	8	41 (61.2)	20.50	22	53.7
GI tissues	0	0	0	0	5	12.2
Fetuses	0	0	0	0	Not applicable	
Newborns	Not applicable				0	0
Total	41	26	67 (100)	33.50	41	100.0
Recovery rate (%)	4.1	2.6		1.7*	4.1*	

*Average percentage recovery rate A1-A2 = Mice dissected before giving birth

B1 = Mice dissected after giving birth GI = Gastrointestinal tissues

M. tissues = Mammary tissues

Table 3.7. Distribution of *Toxascaris leonina* (V. Linstow 1902), larvae in tissues and organs of mice infected during pregnancy and dissected before (n=1) or after (n=1) giving birth.

	Dissected before giving birth		Dissected after giving birth	
Source	A	% Total	B	% Total
Brain	0	0	0	0
Liver	15	21.4	5	12.2
Lung	12	17.1	10	24.4
Uterus	0	0	0	0
Placenta	0	0	Not examined	
M. tissues	0	0	0	0
Heart	1	1.4	0	0.0
Diaphragm	1	1.4	0	0.0
Kidney	0	0.0	1	2.4
Carcass	38	54.3	18	43.9
GI tissues	3	4.3	7	17.1
Fetuses	0	0	Not applicable	
Newborns	Not applicable			
Total	70	100.0	41	100.0
Recovery rate (%)	7.0		4.1	

A = Mice dissected before giving birth B = Mice dissected after giving birth

GI = Gastrointestinal tissues M. tissues = Mammary tissues

The mean distribution (%) of *T. leonina* larvae in tissues and organs of mice infected prior to (n=5 of which 3 were positive) or during (n=2) pregnancy has been summarized as shown in Fig. 3.7. Larval stages were commonly observed in the carcasses, lung and liver.

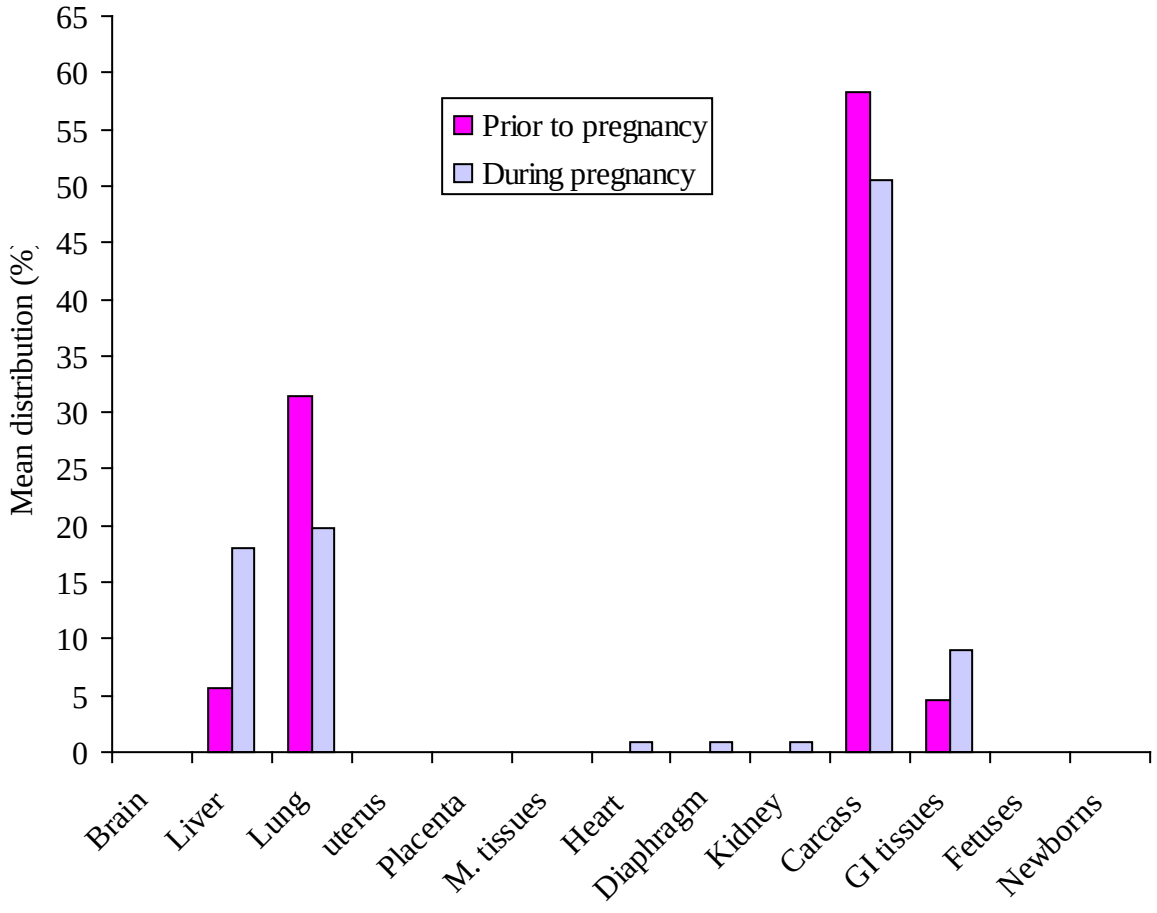


Fig. 3.7. Mean distribution (%) of *Toxascaris leonina* (V. Linstow, 1902) larvae in organs and tissues of mice infected prior to or during pregnancy. GI = Gastrointestinal tissues, M. tissues = Mammary tissues.

3.5 DISCUSSION and CONCLUSIONS

3.5.1 Transmission

Using NIH inbred mice as a model, the possible transplacental and transmammary transmission of *T. canis* larvae from mother to fetus and newborns were demonstrated in the present study. There was, however, a difference in the larval migration pattern in mice infected with *T. canis* prior to or during pregnancy.

Larval stages were not recovered from the uterus, placenta and fetuses in mice infected with *T. canis* prior to pregnancy. Unlike the present study, Lee *et al.* (1976) found *T. canis* larvae in the uterus of pregnant mice infected one week prior to pregnancy. None were, however, found in the placenta or fetuses, corresponding to the findings of the present study. Larval stages were recovered from the newborns in the present study, and this transfer of larvae was the result of transmammary transmission through breast milk, as larval stages were recovered in the mammary tissues of the infected mice.

Larval stages were recovered from the uterus, placenta, fetuses, newborns and mammary tissue in mice infected with *T. canis* during pregnancy. This was in agreement with the results of Lee *et al*. (1976), who infected mice with *T. canis* during pregnancy with the same number of infective eggs as used in the present study, and they recovered larvae of *T. canis* from the fetuses, uterus and placenta of mice dissected prior to giving birth. Tomasovicova, Havasiova-reiterova, Dubinsky and Hovorka (1993) also infected pregnant mice with 1000 infective eggs of *T. canis* each, and reported the intrauterine and lactogenic transfer of *T. canis* larvae. Hence, transplacental transmission of *T. canis*

larvae occurs only if mice are infected during pregnancy. The recovery of *T. canis* larvae form the mammary tissues of mice infected prior to or during pregnancy and in the newborns suggests that there can be transmammary transmission of *T. canis* larvae whether infection is acquired prior to or during pregnancy.

In the present study, larvae were not recovered from the uterus, placenta and fetuses of mice infected with *T. cati* prior to or during pregnancy indicating the absence of intrauterine transmission. However, larval stages were recovered in the mammary tissues and newborns of mice infected prior to or during pregnancy. As with *T. canis*, the recovery of *T. cati* larvae in the mammary tissues and in the newborns indicates transmammary transmission of *T. cati* larvae through breast milk whether infection was acquired prior to or during pregnancy.

In the present study, larval stages were not recovered in the fetuses, newborns, placenta, uterus and mammary tissue in mice infected with *T. leonina* prior to or during pregnancy. This suggests the absence of transplacental and transmammary transmissions of *T. leonina* larvae in mice. However, contrary to this observation, Karbach and Stoye, in 1982, reported transmammary transmission of *T. leonina* in mice experimentally infected either prior to or during pregnancy (Parsons 1987). Transmammary and transplacental transmissions have not been reported in its definitive hosts (Jacobs *et al*. 1994 and Kassai 1999).

The most common route of infection with larvae of *T. canis* in humans is through the ingestion of infective eggs and/or infected meat of paratenic hosts. According to Taylor *et el.* (1996) the transplacental route of transmission could be another possible route of infection in humans, although there is no direct evidence to support this hypothesis. Serological tests would be one way to investigate the possibility of intrauterine transmission. Kincekova, Dubinsky and Pirochova (1995) investigated the transmission of anti-*Toxocara* antibodies from mothers to neonates and found that immunoglobulin G (IgG) antibodies sometimes persisted in babies for as long as six to nine months after birth from anti-*Toxocara* positive mothers. The authors considered this as evidence for the presence of *T. canis* larvae in the neonates, transferred from the infected mothers to the unborn babies through the transplacental route. Taylor *et al*. (1996) after conducting a series of serological studies, however, concluded that the intrauterine route of infection is highly unlikely in humans. They, nevertheless, did not rule out the possibility of other adverse effects, such as miscarriage as a result of new infections during pregnancy.

3.4.2. Larval Distribution

Larval stages were recovered in most tissues and organs of infected mice. This is in agreement with studies conducted by Sprent (1958) and Lee *et al*. (1976). Relatively low numbers of larvae were recovered in the liver and lungs of *T. canis* infected mice during the present study. According to Kayes and Oaks (1976) and Lee *et al*. (1976), most of the larvae of *T. canis* accumulated in the liver and lungs between 24 to 48 hrs after infection from where they gradually migrated to other tissues and organs. The distribution of larvae

to the various tissues and organs of infected mice depends on the time gap between infection and necropsy (Kayes and Oaks 1976).

The majority of larvae were recovered in the carcass and brain of *T. canis* infected mice. The accumulation of larvae in the carcass and brain of infected mice may be related to the long time gap of six to 33 days between infection and necropsy of these mice (Sprent 1958 and Lee *et al*. 1976). The accumulation of larvae in the brain of *T. canis* infected mice might be because of an affinity for the brain (Cox and Holland 1998). According to Oshima (1961b), the larval stages recovered in the intestinal wall may have followed the tracheal route of migration.

The average numbers of *T. canis* larvae recovered from mice infected prior to pregnancy and dissected before or after giving birth were 80 and 70, respectively. In a similar study conducted by Lee *et al*. (1976), comparable mean numbers of larvae (41.76, range 44.66 to 47.0) in one-half of the tissues and organs of pregnant mice infected before pregnancy and dissected before giving birth were reported. The average numbers of larvae recovered in the present study in mice infected during pregnancy and dissected before or after giving birth were, however, 86.8 and 110.3, respectively, which were low when compared to the average numbers of larvae (175, range 158 – 190) recovered in one-half of the tissues and organs of mice infected during pregnancy as reported by Lee *et al*. (1976). The difference in the average recovery of larvae may be related to the number of mice used, the way of infecting the mice and recovering the larval stages. Lee *et al*. (1976) used relatively large numbers of female albino mice (35 mice, each subgroup with

7 mice) and the mice were infected at various intervals of time during pregnancy (5^{th}, 7^{th}, 9^{th}, 11^{th} and 13^{th} days of pregnancy). They examined the infected mice daily for 7 days beginning 24 hrs after infection. Besides these, the variation in the number of larvae recovered from infected mice could arise as a result of irregular inoculations due to the stickiness of the eggs, which may have caused an unknown number of them to remain attached to the wall of the containers, syringes and needles, and the number of eggs passing through the intestine without hatching (Oshima 1961b).

Although no larval stages were recovered from the brain of *T. cati* infected mice, larvae were recovered from most tissues and organs of the infected mice. These larval stages were commonly recovered from the carcasses. The results are in agreement with Sprent (1956) who infected mice with 5000 infective eggs of *T. cati*. He recovered larval stages from the liver, lungs, kidneys, carcass, stomach and intestinal wall. Unlike the present study Sprent (1956) also recovered one to two larval stages in the brain of some of the infected mice. He recovered most larvae from the liver and lungs within 24 to 48 hours post infection, but there was a gradual decrease in numbers recorded later as most of the larvae had migrated to the general tissues and organs of the mice.

In *T. leonina* infected mice, larval stages were commonly recovered from the carcass, lungs and gastrointestinal tissues. According to Dubey (1969) and Sprent (1959), the larvae of *T. leonina* remain in the intestinal epithelium for the first week of infection where they develop before migrating to other tissues. Sprent (1952) indicated that larval stages of *T. leonina* were frequently observed in the liver and lungs in the second week of

post infection. Dubey (1969) studied the migration and development of *T. leonina* larvae by infecting mice with a dose of 2000 embryonated eggs. He recovered larval stages in the lungs, carcass, intestinal wall and intestinal lumen, mesenteric lymph nodes, peritoneal cavity, diaphragm, liver and other abdominal organs. He did, however, not find larval stages in the brain of infected mice. This is in accordance with the findings of the present study. The difference in the numbers of larval stages recovered may have been due to different strains of the parasite used or due to the number of eggs given to the mice (Dubey 1969), or the migration of the larvae into the intestinal lumen and their elimination in the faeces (Sprent 1952).

Transmission through the transplacental or transmammary route in pregnant and lactating mice serves to enhance infection possibilities to the offspring contributing to the survival of the parasites (Shoop and Corkum 1987). The transplacental and/or transmammary modes of transmission of *T. canis* and *T. cati*, combined with the high reproductive potential of mice, may serve as the major distribution mechanism of these parasites, mainly in areas where rodents (Parsons 1987) are a common source of food for stray canids and felines.

In conclusion, based on the outcome of this study, it has been suggested that:

1. There is transplacental transmission of *T. canis* larvae only if mice acquire infection during pregnancy, not if they are infected before pregnancy.
2. There is transmammary transmission of *T. canis* and *T. cati* larvae in mice following infections acquired prior to or during pregnancy,

3. There is neither transplacental nor transmammary transmission of larvae in mice infected with *T. leonina*.
4. Most of the larvae of *T. cati* and *T. leonina* follow the somatic route of migration in mice, but some of them may have follow the tracheal route returning to the intestine and re-entering the tissues as indicated by Oshima (1961a and 1961b) for *T. canis*.

This study has also highlighted the possible role of paratenic hosts in the transmission of ascarid nematodes to dogs and cats.

CHAPTER IV

TOXASCARIS LEONINA: TRANSMISSION TO THE DEFINITIVE HOSTS THROUGH EXPERIMENTALLY INFECTED MICE AS A PARATENIC HOST

4.1 *ABSTRACT*

Transmission of Toxascaris leonina (V. Linstow, 1902) to the definitive hosts, mainly stray dogs and cats, occurs through the ingestion of infected tissues of paratenic hosts. The paratenic host of T. leonina are rodents and they play a significant role as reservoir and transmission to the definitive hosts. The aim of this study was to determine the transmission of T. leonina to dogs and cats through the tissues of experimentally infected mice. Mice were infected with 1000 infective eggs of T. leonina collected from dogs. A total of 10 cats and four dogs were fed infected tissues of mice. Eggs and adult worms were recovered in faecal samples or in necropsies in all the dogs. No eggs or adult worms were, however, recovered in faecal samples in cats that were fed infected mice tissue, using the same procedure and mice as in dogs collected and examined prior to and after treatment with antihelminthics. This study indicated the transmission of T. leonina, originally collected from dogs to dogs and its absence in cats infected with the same source. In conclusion, infection could not be established in cats through the ingestion of tissues of mice infected with T. leonina collected from dogs. Further studies should be conducted to determine cross infection with T. leonina collected from cats. Further more, an investigation has to be conducted to determine the existence of T. leonina strain variation originating from dogs and cats.

Keywords: *Toxascaris leonina, dogs, cats, paratenic host.*

4.2 INTRODUCTION

According to Schantz and Glickman (1978), certain parasitic helminths are capable of infecting a variety of mammalian species that are not the definitive hosts. The larvae usually do not develop to adult worms but may survive indefinitely in the tissues. The transfer of these larval stages from host to host through cannibalism or predation to a susceptible host is a common mode of transmission among the helminth parasites of carnivores (Beaver, Jung and Cupp 1984).

Toxascaris leonina is one of the most common ascarids of domestic and wild carnivores world-wide (Soulsby 1982). Dogs and cats get infected by ingesting embryonated eggs or third-stage larvae encapsulated in the tissues of a paratenic host, which may include rodents, insects, or small carnivores (Sprent and Barrett 1964). The larvae develop to adult worms in the small intestine in the definitive hosts, such as dogs and cats without heart-lung-tracheal migration (Olsen 1974; Read and Skorping 1995). *Toxascaris leonina* infection is prevalent in adolescent or adult dogs and cats (Jacobs *et al*. 1994) due to the acquisition of infection through predation of small mammals. This mode of transmission is considered the most important source of infection of cats (Reinecke 1983; Yamaguchi, MacDonald, Passanisi, Harbour and Hopper 1996) and dogs (Sprent and Barrett 1964; Fisher *et al*. 2002).

When small mammals such as mice get infected with infective eggs of *T. leonina*, the second-stage larvae hatch in the gut and migrate to the various somatic tissues where they moult to third-stage larvae. According to Dunn (1978), this is a true indirect life cycle

with development occurring in the mouse and he considers the mouse as an intermediate host rather than a paratenic host. Intermediate hosts are the main reservoir of *T. leonina* infection and are fully utilized in the life cycle of *T. leonina* (Sprent 1959).

According to Dubinsky *et al.* (1995), small mammals serve as a reservoir for *T. canis* in the wild and predation by canids may play an important role in the circulation and maintenance of the parasite. He furthermore suggests that small mammals are the most important paratenic hosts as the accumulated larvae in their tissues and organs may serve as an important factor in the circulation and maintenance of toxocariasis in the biotopes. The accumulated larvae could possibly serve as good indicators of environmental contamination with *Toxocara* eggs (Dubinsky *et al*. 1995). Similarly, heavier *T. leonina* infections in stray cats (Dubinsky *et al*. 1995; Overgaauw 1997a) and dogs (Sprent and Barrett 1964; Dubinsky *et al*. 1995) have been attributed to predation of small mammals as important food items by these carnivores. O'Sullivan (1997) suggested that stray dogs and cats being food scavengers and/or predators, and usually not treated with anthelminthics would be expected to harbour a heavier worm burden and a wider range of parasites. According to Calvete, Lucientes, Castillo, Estrada, Gracia, Peribanez and Ferrer (1998), stray cats represent potential reservoirs of helminthic infection in domestic cats, especially in rural areas.

Although the genus *Toxascaris* was originally described by Leiper (1907) from a human host, *T. leonina* has never been recorded again from humans (Sprent and Barrett 1964). Hence it is considered to occur in low frequency if at all in visceral larva migrans in

humans (Beaver *et al*. 1984; Roberts and Janovy 2000; Fisher *et al*. 2002), and studies on the life cycle of *T. leonina* usually do not take into account any human involvement.

In spite of its cosmopolitan distribution and infection of both dogs and cats, many more studies were conducted on the prevalence than on the modes of transmission of *T. leonina* to the definitive hosts through infected paratenic hosts. Hence, this study was conducted to determine the transmission of *T. leonina* to the definitive hosts (cats and dogs) through experimentally infected mice with *T. leonina* collected from dogs.

4.3 MATERIALS AND METHODS

4.3.1 Study area and period

This study was conducted at the Animal Unit and Zoology and Entomology Department, University of the Free State, and ClinVet International (Pty) Ltd, Bloemfontein, Republic of South Africa, from April to December 2002.

4.3.2 The Parasite

Adult gravid female *T. leonina* worms were collected from experimentally infected and necropsied dogs. The worms were dissected and eggs were collected from the uteri and washed repeatedly with tap-water through sieves of mesh size 250 and 30 μm. The eggs were cultured in 0.05 mol H_2SO_4 and kept in the dark at room temperature to reach the infective stage.

4.3.3 Infection of mice

Ten mice were each infected with 1000 eggs of *T. leonina* in the first and second experiments. The infected mice were maintained as previously described in the section of modes of transmission (see Section 3.3.3.2). The larvae were allowed 15 days for "growth and development" in the tissues of the mice. The infected mice were dissected on the 15^{th} day. Three of the infected mice from each experiment were dissected and the various tissues were digested using artificial gastric juice. The Baermann method was used to recover and estimate the number of larval stages in the infected mice. The number of larvae recovered from an infected mouse ranged from 98 to 122 (average 108). After determining the mean number of larvae recovered from an infected mouse, the remaining

mice were sacrificed and their tissues individually were, excluding carcasses and skin, minced manually.

4.3.4 Infection of dogs and cats

A total of five cats and two dogs were used in each experiment. The dogs and cats were kept separately in closed environments. They were fed on commercial food. All the experimental dogs and cats were treated with anthelmintics to remove intestinal parasites prior to experimental infection. The dogs and cats were fed minced tissues of mice containing third-stage larvae of *T. leonina* mixed with commercial food (meat or fish) for dogs and cats.

4.3.5 Examination of dogs and cats

Faecal samples of experimental dogs and cats were examined using a saturated salt (NaCl) floatation method to determine the presence of eggs of *T. leonina* from the fifth week of infection onwards (Kassai 1999). The prepatent period of *T. leonina* from infection acquired through eating paratenic hosts is 7 – 11 weeks (Kassai 1999). One of the dogs in the first experimental group died before results were obtained and it was discarded from the study. In the first experiment only faecal examination was done for cats, but the remaining dog was euthanasized and adult worms were recovered from the intestine. In the second experiment, both dogs and cats were treated with an anthelmintic after faecal samples were examined for the eggs of *T. leonina* and the adult worms expelled in the faeces were collected and counted.

4.4 RESULTS

In the first experimental infection of five cats neither eggs nor adult worms were recovered in the faecal samples of the infected cats though the cats were observed continuously for 90 days. In the second experimental infection of five cats, the second-stage larvae were allowed to develop to third-stage larvae in the tissues of mice for some more days. The faecal samples, however, remained negative for eggs or worms in all five infected cats. The cats were also treated with antihelminthics and their faecal samples were examined for worms, but none of them were positive for immature or mature adult stages.

One of the four dogs that were fed *T. leonina* infected tissues of mice, died prior to investigation for parasites. The faecal samples of the remaining three dogs from the first and second experiments were found positive for eggs. The prepatent period for an infected dog was recorded as 50 days.

A number of adult worms were also collected from the dogs. One dog in the first experimental infection of dogs was euthanasized and 20 female and seven male worms were collected. In the second experimental infection of dogs, adult worms were recovered after treating them with an anthelmintic. A total number of six adult female and three male worms from one and 18 female and 14 male worms were collected from the other dog (Table 4.1).

Table 4.1. Number of *Toxascaris leonina* (V. Linstow, 1902) worms recovered from dogs infected through infected tissues of experimentally infected mice.

Dog	Male worm recovered	Female worm recovered	Total
12 CET	14	18	32
25 ODT	3	6	9
CVD 703	7	20	27

CET, ODT and CVD = Codes given to the dogs that were fed tissues of infected of mice.

4.5 DISCUSSION AND CONCLUSIONS

According to Sprent (1959) and Okoshi and Usui (1968), there are two strains of *T. leonina*, namely a feline strain and canine strain. In the present study, the adult worms were originally obtained from infected dogs. This was considered as the canine strain of *T. leonina* although a dog could also be infected by both the feline and canine strains of *T. leonina* (Sprent 1959; Okoshi and Usui 1968).

In spite of the fact that the same batches of eggs were used to infect mice, and the same batches of infected mice tissues were fed to cats and dogs, *T. leonina* eggs and worms were recovered from none of the cats after the first and the second experimental infections. This finding was in agreement with Sprent (1959) who tried to infect cats by feeding them infected tissues of mice. Sprent (1959), however, recovered third-stage larvae in the tissues and fourth-stage larvae in the intestines of cats 35 and 49 days after feeding them the tissues of mice infected with the canine strain of *T. leonina*.

Unlike in the present study, Okoshi and Usui (1968) reported infection of cats with the canine strain of *T. leonina* after feeding them infected mouse tissues. The cats were given an estimated 100 to 1,000 canine strain larvae in tissues of mice. They recovered eggs in faecal samples and adult worms at necropsy performed on the 166th day. They, however, suggested that cats might not be a suitable host for the canine strain of *T. leonina*.

Sprent (1959) and Okoshi and Usui (1968) tried to infect cats by feeding them embryonated eggs of the canine strain of *T. leonina*, but failed and they concluded that infection might not establish in cats through the eggs of the canine strain *T. leonina*.

Sprent (1959) reported infection of cats with the feline strain of *T. leonina* by feeding them infected mouse tissues and he recovered larvae at various developmental stages from the intestinal wall and intestinal contents. Besides this, both Sprent (1959) and Okoshi and Usui (1968) infected cats with infective eggs of the feline strain of *T. leonina* and recovered eggs in the faeces, larvae mainly in the intestinal lumen and intestine wall, and adult worms at necropsy.

Unlike the cats that were fed tissues of mice infected with *T. leonina*, all dogs both in the first and second experiments of the present study were found positive for eggs in their faecal samples and adult worms were recovered during necropsy or after treatment with anthelmintics. This finding was consistent with what Sprent (1959) and Okoshi and Usui (1968) found in dogs experimentally infected by feeding them tissues of mice that were infected with the canine strain of *T. leonina*.

Sprent (1959) infected dogs by feeding them eggs of the feline strain of *T. leonina* and recovered fourth-stage larvae and adult worms only in the intestine. He also infected dogs with eggs of the canine strain of *T. leonina* and recovered larval stages in the intestinal wall and from the intestinal contents of dogs dissected after 21, 32 and 49 days of infection. Similarly, Okoshi and Usui (1968) were able to infect dogs by feeding the tissues of mice infected with the canine strain of *T. leonina* and recovered adult worms.

In the present study nine to 32 adult worms were recovered from dogs infected with *T. leonina* larvae in mouse tissues. Okoshi and Usui (1968) reported 20 - 40 adult worms in

dogs dosed with 10, 000 – 50,000 eggs of the canine strain of *T. leonina*. This low recovery of adult worms might be as a result of the expulsion of larvae in the faeces after they hatched or the release of larvae from tissues into the intestinal lumen.

Besides domesticated animals, Okoshi and Usui (1968) reported the successful infection of dogs with *T. leonina* eggs obtained from the faeces of a lion. The parasites developed to adult worms in the intestines of puppies. They also reported infection of cats with *T. leonina* eggs obtained from the faeces of lion, tiger and cheetah, which developed to adult worms in the intestines of the infected cats.

Though *T. leonina* infects both dogs and cats (Page, Richards, Lewis, Omar & Maizels 1991), reviewed literature showed that *T. leonina* is more often reported from dogs than from cats. *Toxascaris leonina* was reported in domestic cats in the London area (Nichol, Ball and Snow 1981b), in feral cats in London and Sheffield (Nichol, Ball and Snow 1981a), in stray cats in the Netherlands (Overgaauw 1997a), in cats in east central Illinois (Guterbock and Levine 1977), in wildcats in Greece (Papadopoulos *et al*. 1997) and in feral cats in Taiwan (Lin, Bowman, Jacobson, Barr and Giovengo 1990). Positive cases of *T. leonina* infection have not been recorded in the Republic of South Africa from domestic cats (Baker, Lange, Verster & Van Der Plaat 1989) or from domestic and wildcats (Prof. Boomker, J. 2002, personal communication). However, Dr. V. Schwan, Department of Veterinary Tropical Diseases, University of Pretoria, based on his own observations, is certain that *T. leonina* does occur in cats in South Africa (Prof. Dawie. J. Kok, 2003, personal communication).

The reported prevalence rates elsewhere in the world are usually low, in the range of 0.2% to 6% (Guterbock and Levine 1977; Nichol *et al.* 1981a and b; Knaus and Fehler 1989; Lin *et al*. 1990; Papadopoulos *et al*. 1997; Overgaauw 1997a). The highest reported prevalence was 25% (n=4) in necropsies of wildcats in Greece (Papadopoulos *et al*. 1997) and 82% in free-ranging farm cats in the United Kingdom (Yamaguchi *et al*. 1996). However, the prevalence rate of 82% reported in the United Kingdom (Yamaguchi *et al*. 1996) is questionable as all evidence except those reported by Papadopoulos *et al.* (1997) and Yamaguchi *et al*. (1996), suggest that *T. leonina* is less prevalent in cats than in dogs.

In conclusion, infection could not be established in cats fed with the tissues of mice that were infected with *T. leonina* collected from dogs, but dogs were successfully infected from the same source. Further studies should be conducted using modern techniques to explain: 1. why are cats not infected with *T. leonina* that originate from dogs? 2. whether a *T. leonina* strain variation exists or whether the parasite is not just well adapted to infecting cats?

CHAPTER V

EPIDEMIOLOGY OF *TOXOCARA CANIS, TOXOCARA CATI AND TOXASCARIS LEONINA* IN JIMMA, SOUTHWESTERN ETHIOPIA

5.1 *ABSTRACT*

Toxocara canis (Werner, 1782), Toxocara cati (Schrank, 1788) and Toxascaris leonina (V. Linstow, 1902) are roundworms of dogs and/or cats. The study was conducted to determine the epidemiology of these parasites and other intestinal helminths of dogs and cats in the Jimma Town, Southwestern Ethiopia. In a cross-sectional study the following samples were investigated using a saturated sugar floatation method: 436 faecal droppings of dogs from residential areas, roadsides, open fields and playgrounds; 230 faecal samples of dogs taken from the rectum; 77 faecal droppings of cats from households and residential areas and 242 soil samples from residential areas, roadsides, open fields, playgrounds, house dust and dust of dog houses. It was found that 60.78% of faecal samples of dogs from the environment, 78.60% of faecal samples of dogs taken from the rectum, 66.23% of faecal droppings of cats and 33.38% of soil samples were positive for one or more parasites. The parasite species identified were Toxocara canis, T. cati, Toxascaris leonina, hookworms, Spirocerca lupi (Rudolphi, 1801), Trichuris vulpis (Frohlich, 1789), taeniids, Ascaris (Linnaeus, 1758) spp. and/or Strongyloides Grassi, 1879 spp. A relatively high mean egg count of 15.25 with a range of two to 67 was observed for Toxocara canis in dog faecal droppings collected from the environment. This study indicated the presence of Toxocara canis, T. cati, Toxascaris leonina and other helminths in faecal samples of dogs and cats, and soil samples in the study area. Most of the dogs are free-roaming, randomly polluting the environment with

their faeces. This combined with the present low standard of living and hygiene in the country, contributes to a high risk of zoonotic infection.

Keywords: *Epidemiology, faecal examination, prevalence, Toxocara canis, Toxocara cati, Toxascaris leonina, Jimma, Ethiopia.*

5.2 INTRODUCTION

Dogs and cats are the two most common domestic pets that live intimately with man and have a potential role in the direct or indirect transmission of zoonotic helminth parasites to humans (Maetz, Kleinstein, Federico and Wayne 1987; Tan 1997). *Toxocara canis, T. cati* and *T. leonina* are roundworms of dogs and/or cats (Kassai 1999; Radman *et al*. 2000). They are cosmopolitan, mainly prevalent in the tropics and temperate regions (Schantz *et al*. 1980).

It has been estimated that about 4.5 billion of the world's population is infected with helminths (Roberts and Janovy 2000) and according to the World Health Organization (1998), more than one billion people are infected with soil-transmitted helminths. Most of these parasitic diseases occur in the tropics where most countries are poor. Parasitic helminths of domestic pets have been incriminated as a source of disease in humans and animals. Human toxocariasis is an emerging zoonotic disease (Herrmann *et al*. 1985; Robertson, Irwin, Lymbery and Thompson 2000). *Toxocara canis* infections from dogs and *T. cati* infections from cats are the cause of severe diseases in humans and results in economic loss due to the death of the infected animals (Wilcocks and Manson-Bahr 1978).

The risk of toxocariasis in human populations has been associated with the presence of large numbers of dogs in an area or in a community (Mukaratirwa and Taruvinga 1999), the prevalence of parasites in domestic pets, defaecation by pets in the environment, the level of soil contamination (Glickman and Schantz 1981), fecundity of the female worm,

resistance and longevity of the eggs in the environment (Gillespie, Pereira and Ramsey 1991; Jordan *et al*. 1993), as well as the standard of hygiene in the human population (Thompson *et al*. 1986).

The prevalence of *Toxocara* species in domestic pets has been well documented in the developed countries, mainly in the USA, Europe, Japan, Asia and Australia. There is, however, a lack of information on the incidence or prevalence of toxocariasis in Africa in the human population, in the environment and in the definitive hosts (dog and cat) where the burden of helminth parasites is a major health problem.

In Ethiopia, little or no information is available on the prevalence of zoonotic parasitic diseases in domestic pets. In most parts of Ethiopia, domestic pets are an integral part of the household. One or more dogs are kept in the house for the purpose of guarding residential areas (security of property), for hunting, or to herd livestock. Large numbers of dogs (alone or in groups) are commonly seen roaming and scavenging at abattoirs, butcher shops, market places, institutional areas, in residential areas and around playgrounds, etc. Many households that cannot properly feed themselves possess two or more dogs. People are completely unaware that domestic pets could be the source of zoonotic diseases. According to Shimelis (1994), there are no available data that describe the numbers, breed types, management system, and health condition of domestic pets in Ethiopia. He also underlined that the care and treatment given to domestic pets is by far worse than that given to a worn-out shoe, which could be attributed to many factors, amongst them religion, traditions and a lack of public awareness.

Dogs in Ethiopia are seldom treated for parasitic diseases; most of them are strays, have frequent contact with humans and share the same accommodation, and most of them are not properly cared for (Shimelis 1994). Although some attention is given to the importance of dogs as a source of rabies infections (Fekadu 1982), even the vaccination of dogs against rabies is lacking in most parts of the country. Although some studies on parasitic helminths of domestic pets have been conducted in Ethiopia (Mersie 1993; Achenef, Markos, Feseha, Hibret and Tembely 1999), little have been published and there is no proper information on the nature and extent of infection by *Toxocara* species, *T. leonina* and other parasites of domestic pets, or the role of domestic pets in the contamination of the environment, which is considered as the major source of human exposure to infections.

Eggs of helminths such as *Ascaris* (Linnaeus, 1758), *Trichuris* (Roederer, 1761), *Toxocara* and hookworms are considered significant contaminants of soil in the environment (Smith 1998). Soil transmitted helminths are prevalent in Ethiopia, like in other developing countries. In Jimma, southwestern Ethiopia where the present study was conducted, Ali, Mekete and Wodajo (1999) and Haile, Jira and Mola (1994), reported 88.2% and 68.4% prevalence of intestinal parasites among students respectively, with a high prevalence of *Ascaris lumbricoides*, hookworms, and *Trichuris trichiura*. Eggs of *Ascaris* spp. in soil samples from the environment were also reported in Jimma (Kefyalew 1998). The degree of contamination of the environment with the faeces of domestic pets (Dodge 1980) and humans could be a public health hazard.

Given the particular circumstances in relation to domestic pets in Ethiopia, it was hypothesized that evidence of a wide range of parasites might be found in faecal and soil samples from domestic pets. Proper information on the existence and magnitude of parasites of domestic pets can significantly contribute to the planning and management for the control and prevention of animal and human parasitic infections. The aim of this study was to determine the epidemiology of *T.* canis, *T. cati*, and *T. leonina*, as well as and other intestinal parasites in faecal specimens of dogs and cats, and in soil samples collected from the environment. The survey was conducted between November 2001 and January 2002 in Jimma, Southwestern Ethiopia. Recommendations were forwarded for the prevention and control of these parasitic infections in accordance with the outcome of the observations.

5.3 MATERIAL AND METHODS

5.3.1 Study area.

Jimma is located in southwestern Ethiopia, $07^{0}0.4$'N; $36^{0}49$'E, at an altitude of 1740 m. It is situated 330 km south west of Addis Ababa, the capital city of Ethiopia (Fig. 5.1). Jimma has a tropical highland climate characterized by a long wet period, high mean annual rainfall of 1538.30 mm and a mean annual temperature of 18.4^{c} C (Gamatchu 1977).

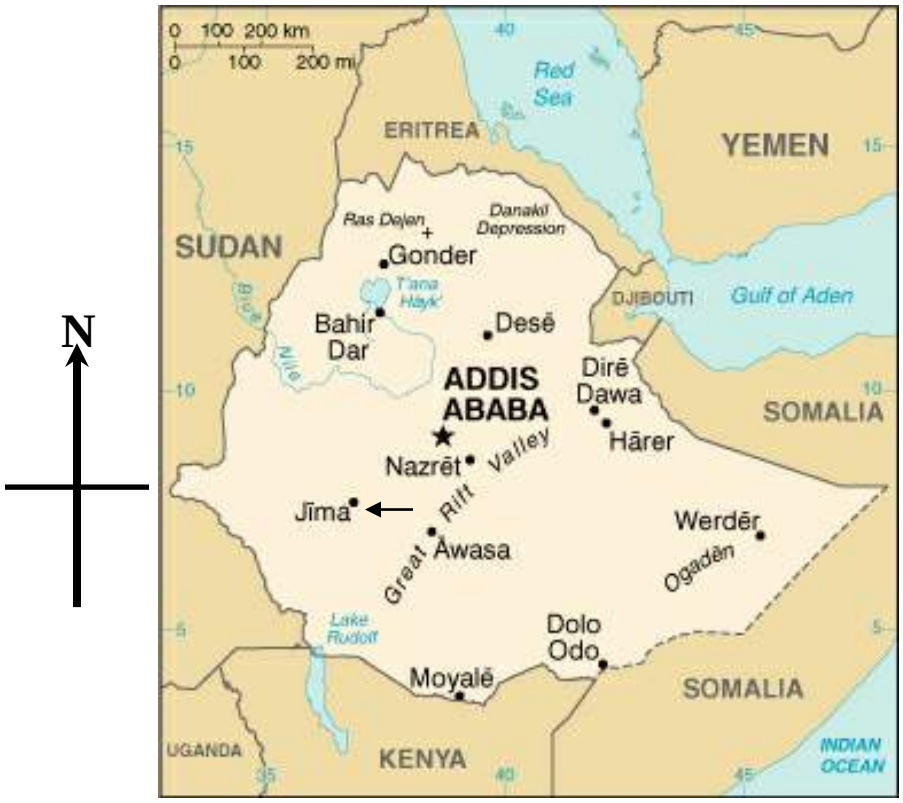


Fig. 5.1. Map of Ethiopia. Arrow indicating Jimma, Southwestern Ethiopia, where the study was conducted.

5.3.2 Study period

This epidemiological study was conducted between November 2001 and January 2002.

5.3.3 Collection of samples

5.3.3.1 Samples of dog faeces

Faecal droppings

A total of 436 faecal droppings of dogs were collected from residential areas, roadsides, open fields, and playgrounds. Dog faeces on the soil surface were identified by appearance, odour (has stinky smell) or by the presence of hairs. From each faecal dropping, a sample of about 2-3 g was collected in a dry, clean and leak-proof, plastic stool container. The containers were labeled with code numbers, type of specimen, place of collection and date of collection. Faecal specimens were collected far apart from each other and the areas were visited only once to avoid sample replications. In some cases pet owners were asked to collect faecal samples.

Rectal faecal samples

A total of 230 faecal samples were collected directly from the rectum of dogs with the aid of a specially designed, smooth, hollow, metallic rod with a rounded end and a groove about 2 cm long near the rounded end (Fig. 5.2). The metallic rod was oiled and inserted gently through the anal opening of a dog to collect about 0.5 to 1.5 g of faecal specimen. The specimen was transferred into a labeled container marked with type of specimen, age of the dog (Young if < 6 months; Adult if ≥ 6 months), date of collection and identification numbers to avoid duplication. Careful attention was given to the collection of faecal samples so that each specimen was from a different source.

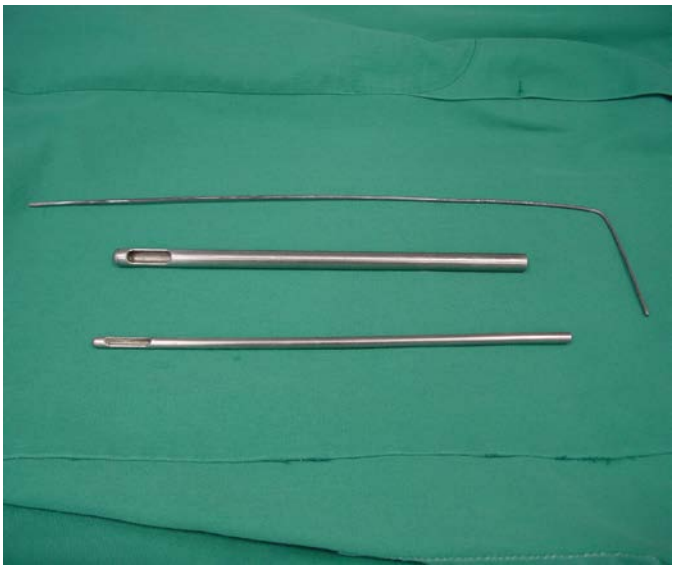


Fig. 5.2. Specially designed, smooth and hollow, metallic rod with a rounded end and a groove at one end that was used to collect faecal samples from the rectum of dogs.

6.3.3.2 Samples of cat faeces

A total of 77 samples of cat faeces were collected only from households and residential areas. Approximately 1 to 2.5 g of cat faeces was collected for each sample. It was often difficult to collect cat faeces since a cat buries its faeces after defecation. When specimens were not collected during a visit, containers were given to the cat owners and samples were collected the next day.

5.3.3.3 Soil samples

A total of 242 soil samples were collected from residential areas, roadsides, open fields, playgrounds and dust from houses and doghouses. Soil samples from the environment were randomly chosen from areas where faecal contamination from dogs and cats could be concentrated and where human contact with potentially contaminated soil was most

likely. In the case of house dust and dust from doghouses, samples were collected by scraping or sweeping the areas (most were not cemented). Great care was taken to avoid collection of soil samples containing faeces of dogs, cats or humans. Approximately 10 to 15 g of soil was collected from the uppermost surface at each location. Samples were taken to the laboratory in dry and clean plastic containers. Figure 5.3 shows a typical location with dogs and children, where faecal and soil samples were collected.

Fig. 5.3. Faecal samples collected from these dogs were found positive.

5.3.3.4 Ethical issues

An official letter from the Research and Publication Office of the Jimma University was written to the concerned individuals and bodies. The objectives of the study were explained to the domestic pet owners. The method used to collect faecal samples from the rectum of the dogs was not invasive. When a domestic pet was diagnosed positive, the

owners were informed about the result of laboratory investigations and advised to take their pets to the veterinary clinic.

5.3.4 Parasitological laboratory procedures.

Faecal specimens of dogs and cats, and soil samples brought to the laboratory were examined using 40% sucrose solution (400 g sugar and 600ml H_2O) as a direct floatation technique, and parasite eggs were identified microscopically (Kassai 1999). Positive control samples were run periodically to avoid false positive observations. Relative egg counts of parasite species were obtained by counting the number of eggs present under the coverslip, a non-quantitative method. The laboratory where faecal and soil samples were examined is shown in Figure 5.4.

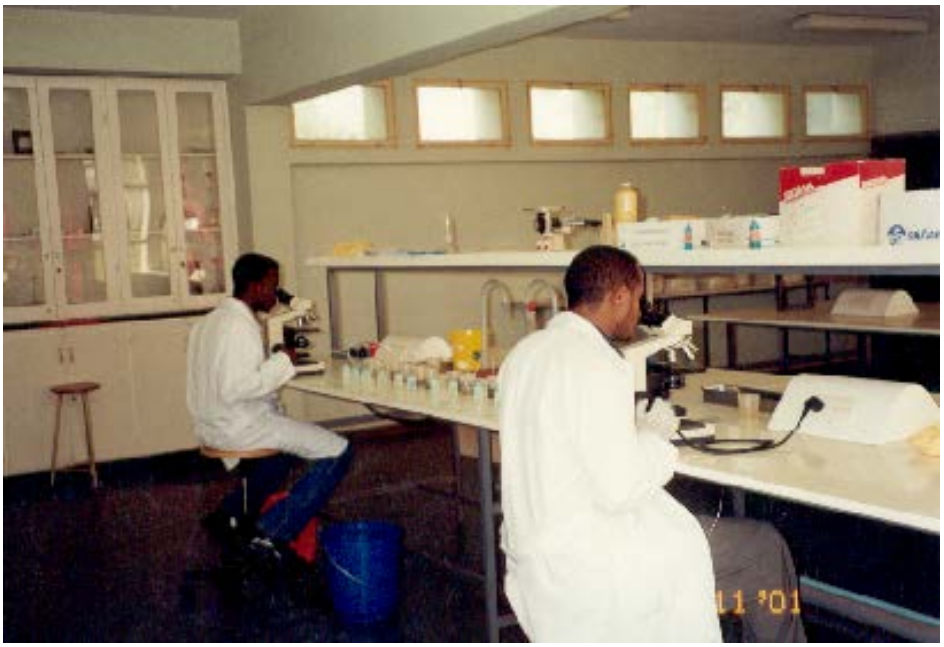


Fig. 5.4. Laboratory examination of faecal specimens of dogs, cats and soil samples for parasites at Jimma University, in School of Medical Laboratory Technology.

5.3.4.1 Dog or cat faeces

Each faecal sample in the plastic container was thoroughly mixed with the aid of an applicator stick. About 1 g of faecal specimen was transferred to a faecalizer tube. Sucrose solution (40%) was added up to a specific mark (indicated with an arrow) and the sample was properly mixed with the solution in the faecalizer tube. After tightening the porous lid of the faecalizer tube, it was filled to the brim with the sucrose solution. A clean coverslip was placed on the meniscus without trapping air beneath it. The preparation was left for about 10 to 15 minutes, before the coverslip was lifted, transferred to a glass slide and examined for the presence of parasite eggs using a compound microscope (10X objective). Dried or hard faecal samples were soaked in sucrose solution for 5 to 10 minutes to soften prior to processing.

6.3.4.2 Soil samples

The soil samples were passed through a series of five sieves of 1000, 500, 300, 90 and 45 µm mesh sizes to remove fine dust, stones, grass, sand and other small or larger debris. About 3 to 5 g soil samples was taken from the 45 µm sieve and placed in a beaker. To each gram of soil sample, 2 ml of dilute sodium hypochlorite (3 parts bleaching fluid to 10 parts water) was added as a wetting agent before being homogenized (WHO 1967). The mixture was homogenized with an applicator stick. About 1 g homogenized wet soil sample was transferred into a faecalizer tube. The faecalizer tube was half filled with sucrose solution and the sample was mixed, then the tube was filled to the brim. A coverslip was placed gently on top of the tube and left for about 10 to 15 minutes. The coverslip was then transferred to a glass slide and examined for the presence of parasite

eggs using a compound microscope (10X objective). No attempt was made to distinguish between *T. canis* and *T. cati* eggs as they cannot be distinguished by light microscopy (Uga *et al*. 1996; Ruiz de Ybanez, Garijo and Alonso 2001).

5.3.5 Statistical analyses

Descriptive statistics and chi-square tests were used for statistical analysis. Prevalence of parasites was determined using the following formula (Bland 1987; Kassai 1999):

$$\text{Prevalence} = \frac{\text{Number of host individuals infected with a particular parasite spp.}}{\text{Number of host individuals examined}} \; x\,100$$

5.4 RESULTS

5.4.1 Faecal samples of dogs collected from the environment

A total of 436 dog faecal samples were collected from the environment. Of these, 208 (47.71%) were collected in residential areas, 171 (39.22%) along roadsides, 48 (11.01%) in open fields and 9 (2.06%) in playgrounds (Table 5.1). Faecal examination, with a non-quantitative floatation method showed that helminth eggs were present in an average of 60.78% of the samples. The percentages of the samples containing eggs were closely similar for the different locations at which samples were collected, varying between 55.56% (playgrounds) and 65.38% (residential areas) (Table 5.1).

Although most (47.02%) of the positive samples were for a single parasite species, several were positive for two (10.09%), three (3.21%), four (0.23%) or even five (0.23%) parasites (Table 5.2). Hookworm eggs were the most abundant occurring in 36.24% of the samples. This was followed by *T. canis* (20.18%), *Ascaris* sp. (8.49%), *S. lupi* (4.59%), *T. vulpis (3.44%), T. leonina* (3.44%), taenids (1.38%) and *Strongyloides* sp. (1.15%) (Fig. 5.5). The highest prevalence of *T. canis* (33.33%; Table 5.3), hookworms (45.83%; Table 5.4) and *T. leonina* (4.33%; Table 5.5) were found in faecal samples collected from playgrounds, open fields and residential areas, respectively. The overall average mean egg count was 11.88 (range 2 - 67) eggs per slide. The highest relative egg count was for *T. canis* (15.25). This was followed by *S. lupi* (12.80), *Ascaris* sp. (12.35), hookworms (11.02), *T. leonina* (11.00), *Strongyloides* sp. (5.80), *T. vulpis* (5.07) and taenids (3.17) (Table 5.6).

Table 5.1.Helminth eggs recovered from samples of dog faeces collected in the environment. Total number of samples collected = 436.

			Helminth eggs found	
Site	Number (n)	% Total	Yes	% Positive
Residential areas	208	47.71	136	65.38
Open fields	48	11.01	27	56.25
Road sides	171	39.22	97	56.73
Playgrounds	9	2.06	5	55.56
Total	436	100.00	265	60.78

Table 5.2. Prevalence of single and multiple parasite eggs found in 265* of 436 samples of dog faeces collected in the environment.

Type	Samples (n)	% Total
Single infection	205	47.02
Double infections	44	10.09
Triple infections	14	3.21
More than triple infections	2	0.46

* Number of positive samples.

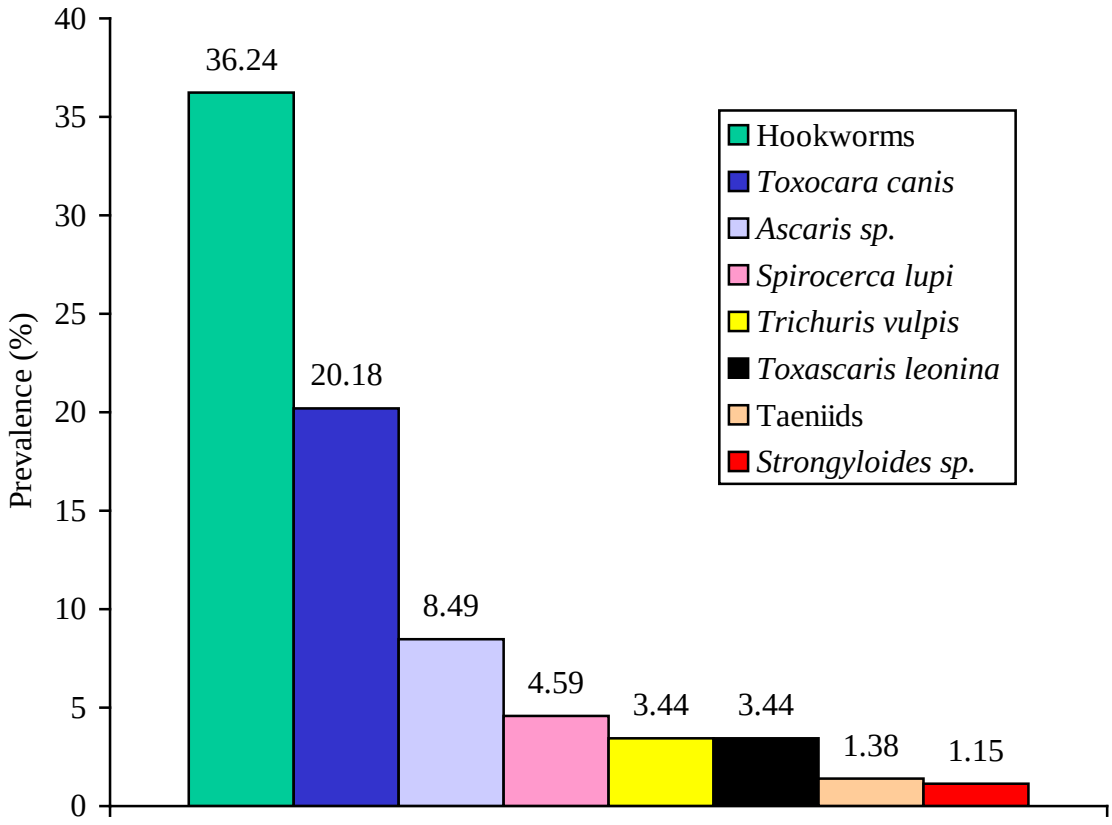


Fig. 5.5. The prevalence of different parasite species identified from eggs in 265* of 436 samples of dog faeces collected from the environment. (* Some faecal samples contained more than one parasite species).

Table 5.3. *Toxocara canis* (Werner, 1782) in samples of dog faeces collected from the environment.

Site	Samples (n)	Positive (n)	% Positive
Residential areas	208	48	23.08
Open fields	48	5	10.42
Road sides	171	32	18.71
Playgrounds	9	3	33.33
Total	436	88	20.18

Table 5.4. Hookworm eggs in samples of dog faeces collected from the environment.

Site	Samples (n)	Positive (n)	% Positive
Residential areas	208	72	34.62
Open fields	48	22	45.83
Road sides	171	62	36.26
Playgrounds	9	2	22.22
Total	436	158	36.24

Table 5.5. *Toxascaris leonina* (V. Linstow, 1902) eggs in samples of dog faeces collected from the environment.

Site	Samples (n)	Number	% Positive
Residential areas	208	9	4.33
Open fields	48	0	0.00
Road sides	171	6	3.51
Playgrounds	9	0	0.00
Total	436	15	3.44

Table 5.6. Relative egg counts of parasite species identified in samples of dog faeces collected from the environment.

Parasite species	Positive (n)*	Egg count (%)	Range	Mean
Hookworms	158	1741 (42.62)	2 - 43	11.02
Toxocara canis	88	1342 (32.85)	2 - 67	15.25
Ascaris sp.	37	457 (11.19)	3 - 28	12.35
Spirocerca lupi	20	256 (6.27)	3 - 33	12.80
Toxascaris leonina	15	165 (4.04)	3 - 22	11.00
Trichuris vulpis	15	76 (1.86)	2 - 13	5.07
Strongyloides Sp.	5	29 (0.71)	1 - 16	5.80
Taenids	6	19 (0.47)	2 - 6	3.17
Total	344	4085 (100.00)	2 - 67	11.88

* Some faecal samples contained more than one parasite species.

5.4.2 Faecal samples collected from the rectum of dogs

Of the 230 dogs examined for the presence of parasites in the faecal samples collected from the rectum, 181 (78.70%) were positive. The infection rate of young dogs (79.31%) was similar to that of adult dogs (77.65%). Similar infection rates were also observed in female (79.41%) and male (78.13%) dogs (Table 5.7).

Most dogs (48.70%) were infected with a single parasite species. Some dogs were also infected with two (25.22%), three (3.48%) and more than three (1.3%) parasites (Table 5.8). Hookworm eggs had the highest prevalence of 38.70%. This was followed by *T. canis* (30.87%), *T. leonina* (6.96%), *S. lupi* (5.65%), *T. vulpis* (3.91%), *Ascaris* sp. (3.48%), *Strongyloides* sp. (2.17%), and the least were taenids (1.74%) (Fig. 5.6).

As shown in Table 5.9, using the chi-square test, the prevalence of *T. canis* was significantly higher ($\chi^2 = 29.09$; $P < 0.001$, chi-square test) in young dogs (43.45%) than in the adult dogs (9.41%). The prevalence of *T. canis* was significantly higher ($\chi^2 = 15.07$; $P < 0.001$, chi-square test) in female (44.12%) than in male (20.31%) dogs. The prevalence of hookworm eggs was not significant when comparing adult (43.53%) to young (35.86%) dogs ($\chi^2 = 1.33$; $P > 0.05$, chi-square test), but the prevalence was significantly higher in female (40.20%) than in male dogs (37.50%) dogs ($\chi^2 = 17.96$; $P < 0.001$, chi-square test; Table 5.10). The prevalence of *T. leonina* was similar for young (6.21%) and adult (8.24%) dogs, which was also true for female (6.86%) and male (7.03%) dogs (Table 5.11). The highest relative egg count was for *T. canis* (42.89%)

with a mean egg count of 11.38 (range 3 - 31). This was followed by egg counts of *T. leonina* (9.13), hookworms (8.40) and the least was for taenids (2.75) (Table 5.12).

Table 5.7. Parasite infections in samples of dog faeces collected from the rectum.

		Number (n)	% Of Total	Helminth eggs found	
				Yes	% Positive
Age	Young	145	63.04	115	79.31
	Adult	85	36.96	66	77.65
Sex	Female	102	44.35	81	79.41
	Male	128	55.65	100	78.13
	Total	230	100.00	181	78.70

Table 5.8. Prevalence of single and multiple helminth parasite eggs found in 181* of 230 samples of dog faeces collected from the rectum.

Type	Samples (n)	% Total
Singe infection	112	48.70
Double infections	58	25.22
Triple infections	8	3.48
More than triple infections	3	1.30

* Number of positive samples.

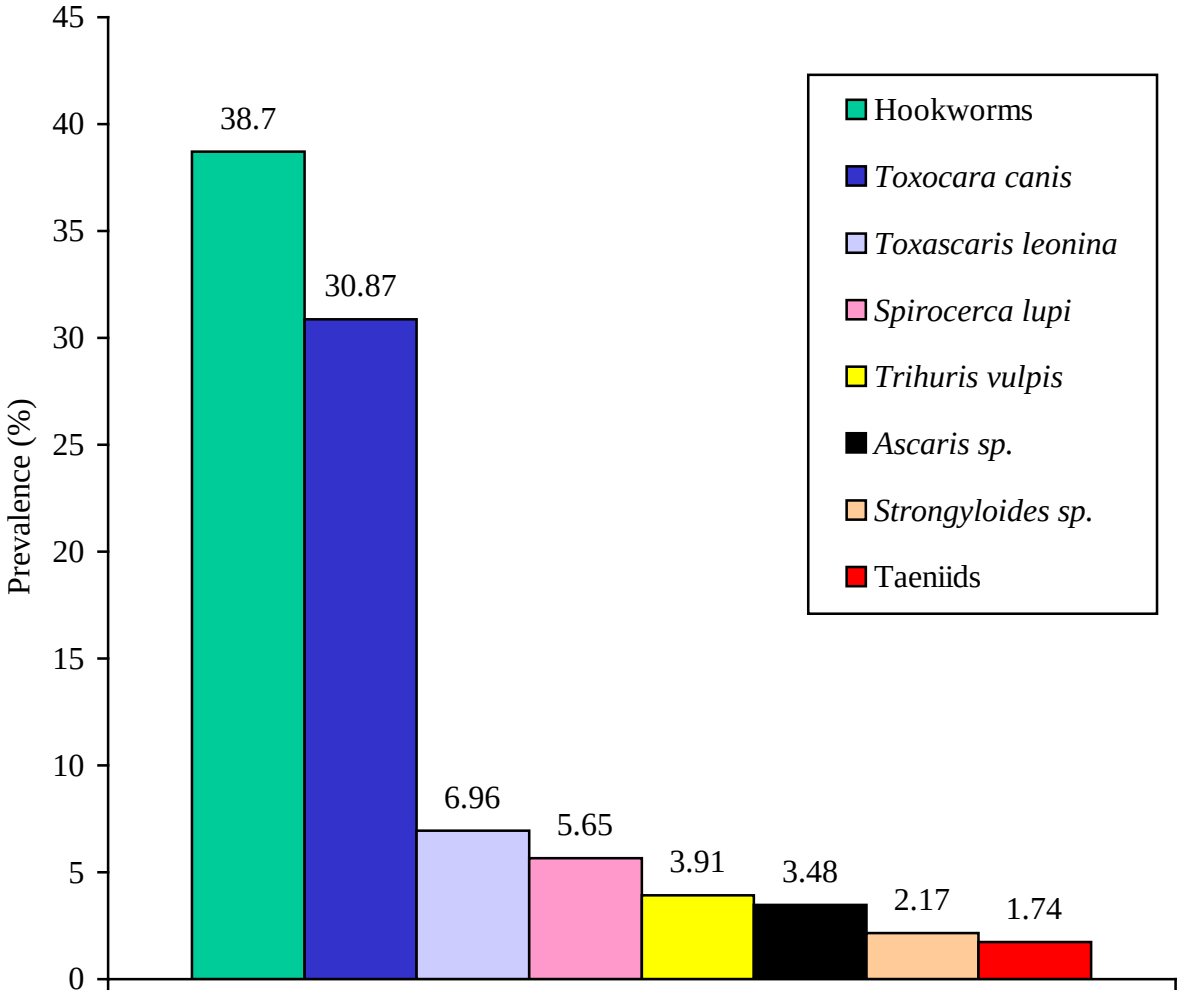


Figure 5.6. The prevalence of different parasite species identified from eggs in 181* of 230 samples of dog faeces collected from the rectum. (* Some faecal samples contained more than one parasite species).

Table 5.9. *Toxocara canis* (Werner, 1782) eggs in samples of dog faeces collected from the rectum.

		Samples (n)	Positive (n)	% Positive
Age*	Young	145	63	43.45
	Adult	85	8	9.41
Sex**	Female	102	45	44.12
	Male	128	26	20.31
	Total	230	71	30.87

* $\chi^2 = 29.09$, d.f. = 1, $P < 0.001$; ** $\chi^2 = 15.07$, d.f. = 1, $P < 0.001$.

Table 5.10. Hookworm eggs in samples of dog faeces collected from the rectum.

		Samples (n)	Positive (n)	% Positive
Age*	Young	145	52	35.86
	Adult	85	37	43.53
Sex**	Female	102	41	40.20
	Male	128	48	37.50
	Total	230	89	38.70

* $\chi^2 = 1.33$, d.f. = 1, $P > 0.0$; ** $\chi^2 = 17.96$, d.f. = 1, $P < 0.001$.

Table 5.11. *Toxascaris leonina* (V. Linstow, 1902) eggs in samples of dog faeces collected from the rectum.

		Samples (n)	Positive (n)	% Positive
Age	Young	145	9	6.21
	Adult	85	7	8.24
Sex	Female	102	7	6.86
	Male	128	9	7.03
	Total	230	16	6.96

Table 5.12. Relative egg counts of parasite species identified in samples of dog faeces collected from the rectum.

Parasite species	Positive (n)*	Egg count (%)	Range	Mean
Toxocara canis	71	808 (42.89)	4 - 31	11.38
Hookworms	89	748 (39.70)	2 - 25	8.40
Toxascaris leonina	16	146 (7.75)	2 - 17	9.13
Spirocerca lupi	13	67 (3.56)	3 - 12	5.15
Ascaris sp.	8	51 (2.71)	3 - 12	6.38
Trichuris vulpis	9	29 (1.54)	2 - 5	3.22
Strongyloides sp.	5	24 (1.27)	2 - 10	4.80
Taeniids	4	11 (0.58)	1 - 5	2.75
Total	215	1884 (100.00)	1 - 31	8.76

* Some faecal samples contained more than one parasite species.

5.4.3 Samples of cat faeces from household and residential areas

A total of 77 samples of cat faeces was collected from households and residential areas. Helminth eggs were recovered from 51 (66.23%) of the samples (Table 5.13). A higher prevalence (71.67%) of helminth parasites was recorded for faecal samples collected in households compared to residential areas (47.06%) (Table 5.13). Most (84.31%) faecal samples had only a single parasitic infection and only 15.67% of the samples showed infection with two parasites (Table 5.14). The only parasites identified were *T. cati* with the highest prevalence (67.80%), followed by hookworms (19.48%) and *T. leonina* (5.19%) (Fig. 5.7). A higher prevalence of *T. cati* eggs was observed in faeces collected from households (61.67%) compared to residential areas (17.65%) (Table 5.15). Hookworm eggs were detected in 20% and 17.65% of faecal samples collected from households and residential areas, respectively (Table 5.16). The prevalence of *T. leonina* in households (5%) and residential areas (5.88%) was similar (Table 5.17). The highest relative egg count was that of *T. cati* with a mean egg count of 17.60 (range 5 - 44), followed by hookworms (10.86), and *T. leonina* (8.05) (Table 5.18).

Table 5.13. Helminth eggs recovered from samples of cat faeces collected from households and residential areas. Total number of samples = 77.

			Helminth eggs found	
Source	Samples (n)	% Total	Yes	% Positive
Household	60	77.92	43	71.67
Residential area	17	22.08	8	47.06
Total	77	100.00	51	66.23

Table 5.14. Prevalence of single and multiple helminth parasite eggs found in 51* of 77 samples of cat faeces collected from households and residential areas.

Type	Samples (n)	% Total
Singe infection	43	84.31
Double infections	8	15.69

*Number of positive samples.

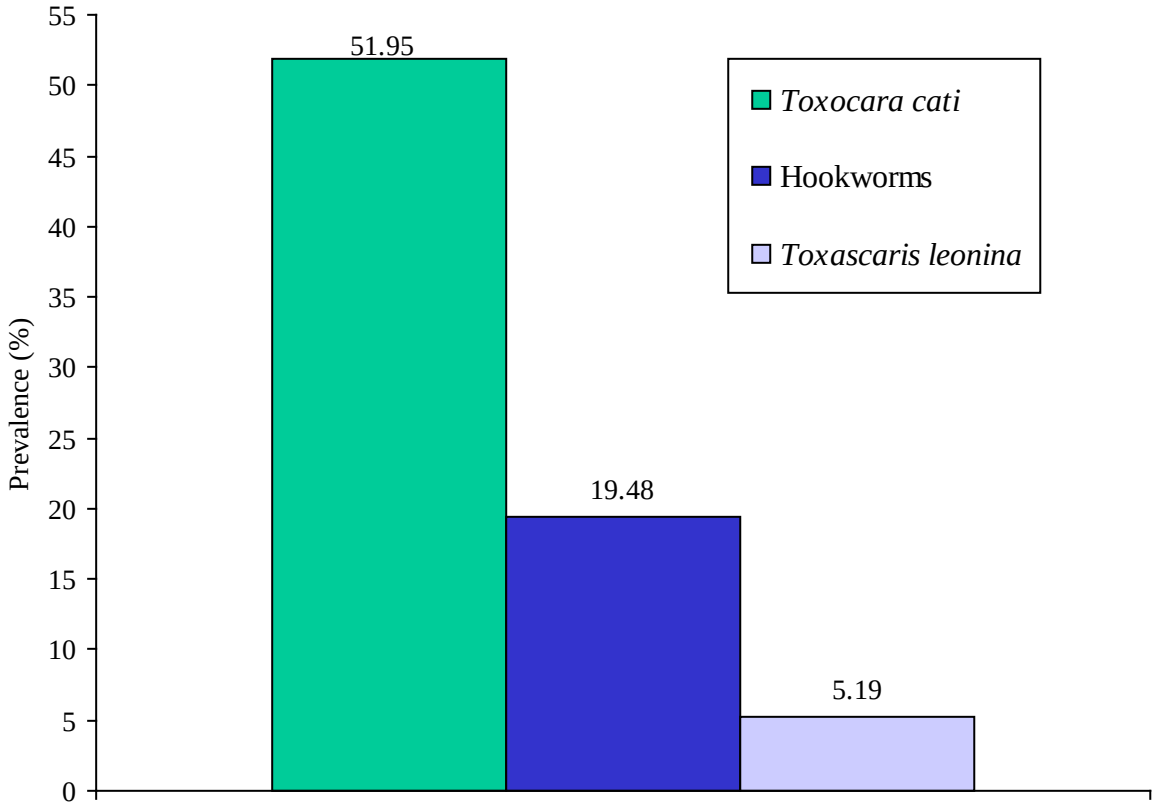


Fig. 5.7. The prevalence of different parasite species identified from eggs in 51* of 77 samples of cat faeces collected in the households and residential areas. (* Some faecal samples contained more than one parasite species).

Table 5.15. *Toxocara cati* (Schrank, 1788) in samples of cat faeces collected from households and residential areas.

Site	Sample (n)	Positive (n)	% Positive
Household	60	37	61.67
Residential area	17	3	17.65
Total	77	40	51.95

Table 5.16. Hookworm eggs in samples of cat faeces collected from households and residential areas.

Site	Sample (n)	Positive (n)	% Positive
Household	60	12	20.00
Residential area	17	3	17.65
Total	77	15	19.48

Table 5.17. *Toxascaris leonina* (V. Linstow, 1902) eggs in samples of cat faeces collected from households and residential areas.

Site	Sample (n)	Positive (n)	% Positive
Household	60	3	5.00
Residential area	17	1	5.88
Total	77	4	5.19

Table 5.18. Relative egg counts of parasite species identified in samples of cat faeces collected from households and residential areas.

Parasites	Positive (n)*	Egg count (%)	Range	Mean
Toxocara cati	40	704 (78.05)	5 - 44	17.60
Hookworms	15	163 (18.07)	3 - 31	10.86
Toxascaris leonina	4	35 (3.88)	7 - 13	8.75
Total	59	902 (100.00)	3 - 44	15.3

* Some faecal samples contained more than one parasite species.

5.4.4 Soil samples

A total of 242 soil samples from various localities was collected and examined for the presence of parasite eggs. Of these, 141 (58.26%) were collected in residential areas, 28 (11.57%) as house dust, 24 (9.92%) in open fields, 23 (9.50%) along roadsides, 14 (5.79%) in playgrounds and 12 (4.94%) in doghouses. An overall prevalence of 33.88% was observed. The contamination of soil with parasite eggs was the highest for dust collected from doghouses (83.33%) and the least for open fields (25%) (Table 5.19). Most soil samples were contaminated with the eggs of a single parasite species (81.71%). Contamination with two parasites was observed in 18.29% of the samples (Table 5.20). The parasites identified in soil samples were *Toxocara* spp. (14.05%), hookworms (9.50%), *Ascaris* sp. (9.09%), *Toxascaris leonina* (4.13%), *Trichuris* sp. (2.48%) and *S. lupi* (0.83%) (Fig. 5.8). The prevalence of *Toxocara* spp. eggs varied from 8.33% (open fields) to 58.33% (dust from doghouses) (Table 5.21). Most eggs of *Toxocara* spp. were embryonated and the larvae within the embryonated eggs were motile indicating that they were viable and infective. Hookworm eggs were common in soil samples collected from house dust (14.29%), playgrounds (14.29%) and open fields (16.67%) (Table 5.22). *T. leonina* eggs were collected in only 4.13% of the soil samples and no eggs were recovered in soil samples from open fields and playgrounds (Table 5.23). The highest relative egg count was for *Ascaris* spp. with a mean egg count of 4.14 (range 1 - 7). This was followed by egg counts of *T. leonina* (3.60), *Toxocara* spp. (3.53), *S. lupi* (3.50) and the least was for *T. vulpis* (2.50) (Table 5.24).

Table 5.19.Helminth eggs recovered from soil samples collected from the environment. Total number of samples collected = 242.

			Helminth eggs found	
Site	Number (n)	% Total	Yes	% Positive
Residential areas	141	58.26	43	30.50
House dust	28	11.57	12	42.86
Open fields	24	9.92	6	25.00
Road sides	23	9.50	6	26.09
Playgrounds	14	5.79	5	35.71
Dust from dog houses	12	4.96	10	83.33
Total	242	100.00	82	33.88

Table 5.20. Prevalence of single and multiple parasite species eggs found in 82* of 242 soil samples collected from the environment.

Type	Samples (n)	% Total
Singe infection	67	81.71
Double infections	15	18.29
Total	82	100.00

*Number of positive samples.

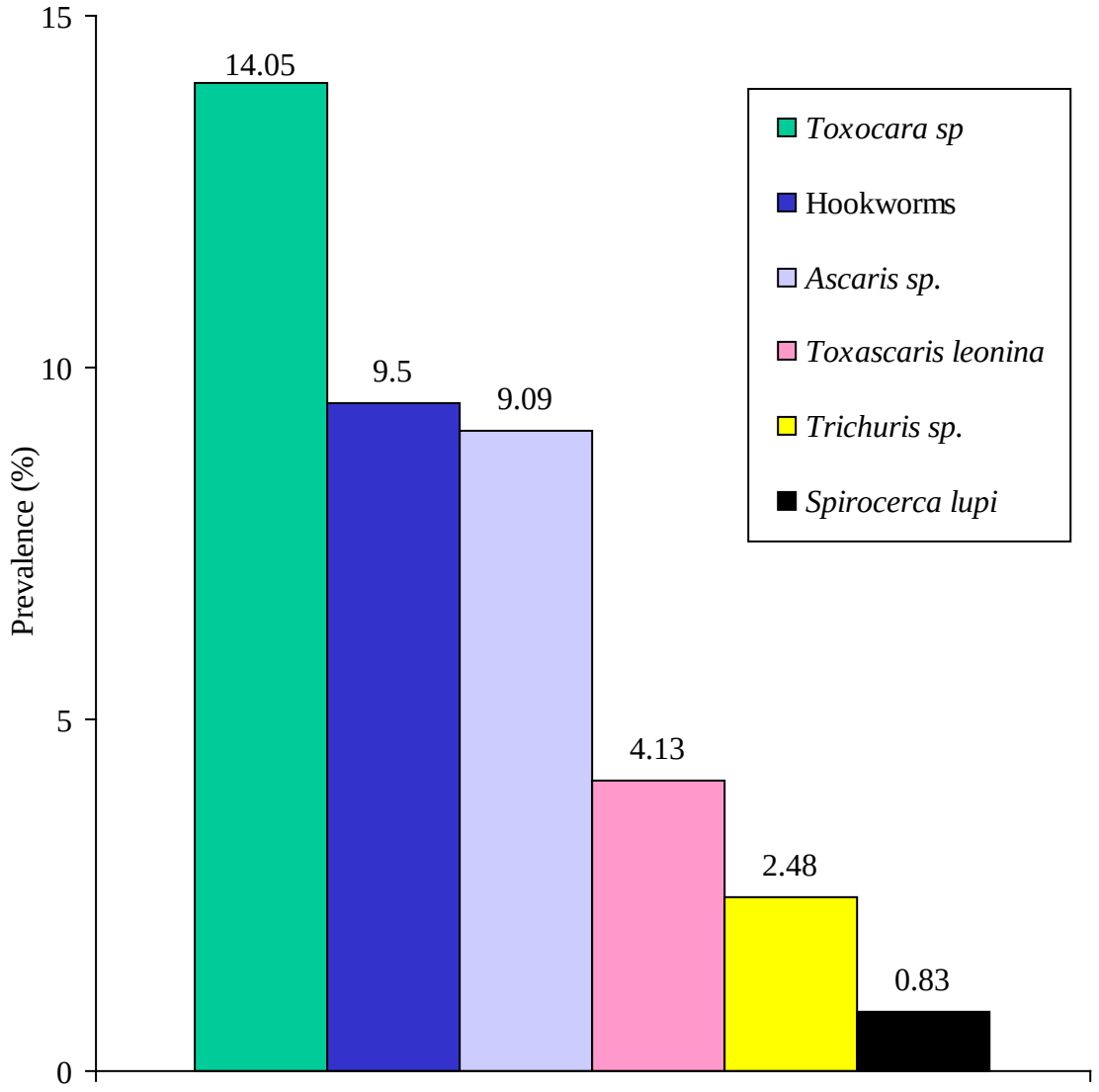


Fig. 5.8. The prevalence of different parasite species identified from eggs in 84 of 242 soil samples collected from the environment. (* Some soil samples contained more than one parasite species).

Table 5.21. *Toxocara* Stiles, 1905 spp. eggs in soil samples collected from the environment.

Site	Sample (n)	Positive (n)	% Positive
Residential areas	141	17	12.06
House dust	28	4	14.29
Open fields	24	2	8.33
Road sides	23	2	8.70
Playgrounds	14	2	14.29
Dust from dog houses	12	7	58.33
Total	242	34	14.05

* Some soil samples contained more that one parasite species.

Table 5.22. Hookworm eggs in soil samples collected from the environment

Site	Samples (n)	Positive (n)	% Positive
Residential areas	141	9	6.38
House dust	28	4	14.29
Open fields	24	4	16.67
Road sides	23	3	13.04
Playgrounds	14	2	14.29
Dust from dog houses	12	1	8.33
Total	242	23	9.50

* Some soil samples contained more that one parasite species.

Table 5.23. *Toxascaris leonina* (V. Linstow, 1902) eggs in soil samples collected from the environment.

Site	Samples (n)	Positive (n)	% Positive
Residential areas	141	5	3.55
House dust	28	2	7.14
Open fields	24	0	0.00
Road sides	23	1	4.35
Playgrounds	14	0	0.00
Dust from dog houses	12	2	16.67
Total	242	10	4.13

Table 5.24. Relative egg counts of parasite species identified from samples of soil collected from the environment.

Parasite species	Positive (n)*	Egg count (%)	Range	Mean
Toxocara spp.	34	120(34.99)	1 - 7	3.53
Ascaris sp.	22	91(26.53)	1 - 7	4.14
Hookworms	23	74(21.53)	1 - 6	3.13
Toxascaris leonina	10	36(10.50)	2 - 7	3.60
Trichuris vulpis	6	15(4.37)	2 - 3	2.50
Spirocerca lupi	2	7(2.04)	2 - 5	3.50
Total	97	343(100.00)	1 - 7	3.54

*Some faecal samples contained more than one parasite species.

5.5 DISCUSSION AND CONCLUSIONS

The results of this study confirmed the presence of *T. canis, T. cati, T. leonina* and other intestinal parasites in dogs and cats in the study area, indicating a potential danger to humans. The overall prevalence rates were 33.88% in soil samples, 60.78% in faecal droppings of dogs in the environment, 66.23% in samples of cat faeces collected from the residential areas and households and 78.7% in samples of dog faeces from the rectum. The most frequently occurring parasites in the examined faecal and soil samples were hookworms and *Toxocara* species.

The actual prevalence of some parasitic infections in dogs, cats and soil samples may, however, be even higher than determined in this study. Some of the factors that could have affected the findings are the limitations of the methods used, intermittent egg shedding (Kirkpatrick 1988), seasonal variations, environmental conditions, the choice of sampling sites (Surgan, Colgan, Kennett and Paffmann 1980; Childs 1985), light infections and the presence of only male worms in the host (Overgaauw 1997a).

Dog faecal deposits

As no previous studies had been done on faecal deposits collected from the environment or rectal samples in Ethiopia, the results were compared to similar studies conducted in other countries. The overall prevalence of 60.78% infection rate in samples of dog faeces collected from the environment was comparable to 58% observed in fresh faecal deposits of well-cared-for dogs examined using a formol-ether concentration technique in Jamaica (Robinson, Thompson and Lindo 1989) and 73% in dog faecal droppings collected from

the roadsides and public places examined, using a combination of direct smear and formalin-ethyl acetate sedimentation, and Sheather's sugar floatation in Egypt (Abou-Eisha and Abdel-Aal 1995). Jaskoski, Barr & Borges (1982), using a sugar floatation technique, reported between 13.7% and 71% infection in dog faecal samples collected from residential areas and from dogs in the Animal Care and Control Center in Chicago. According to Jaskoski *et al*. (1982), the high prevalence of parasite eggs in dog faecal samples was related to the number of infected dogs and failure to enforce regulations requiring disposal of dog faeces by dog owners.

The overall prevalence of 60.78% parasite infections in samples of dog faeces collected from the environment was very high when compared to surveys conducted by Overgaauw (1997a) in the Netherlands. He reported a prevalence of 2.9% faecal samples of household dogs that were examined using a $ZnSO_4$ floatation method. Using the same diagnostic method, Overgaauw and Boersema (1998b) reported a prevalence of 18% nematode infection in dog' faecal samples collected from dog breeding kennels in the Netherlands. They have suggested that the low prevalence of nematode eggs in dog faecal samples collected from households was due to increased availability of better anthelmintics, high/regular deworming frequency by owners, awareness of possible worm problems, more responsibility of pet ownership and/or the absence or rare occurrence of stray dogs in the Netherlands. But the higher prevalence of nematode eggs in dog faecal samples collected from dog breeding kennels compared to those from households was suggested to be as a result of infection pressure in kennels where the closed environment increased the risk of infection.

Faecal samples from the rectum of dogs

The overall prevalence of 78.7% helminths based on faecal samples taken from the rectum of dogs in the present study was comparable to the 76% infection rate in faecal samples collected per rectum in stray dogs following euthanasia and examined using Kyron® faecalizer tubes in Bloemfontein, South Africa (Minnaar, W.N., Krecek, R.C. and Fourie, L.J. 2002). These findings are higher compared to 52.19% parasitic infection found in stray dog faecal samples taken from the rectum and examined using a saturated salt (NaCl) floatation technique in Turkey (Doganay and Oge 1993). It was, however, relatively low compared to the 100% incidence of helminth infections found in dogs examined with adhesive tape swabs and fresh faecal samples collected per rectum using a qualitative faecal floatation method in Jericho and Zuurbekom in South Africa (Minnaar, Krecek and Rajput 1999). Bugg, Robertson, Elliot and Thompson (1999) reported a 28.7% (range, 5.3 to 51.0%) parasitic infection in faecal samples of dogs from pet shops, refuges, breeding kennels, veterinary clinics and exercise areas in Australia and examined using a sedimentation technique in water followed by centrifugal floatation in saturated zinc sulfate solution. The differences in prevalence may be explained by the different method used to examine faecal samples and the condition of the dogs (stray or sick) examined. According to Okoshi and Usui (1967b), as a faecal sample has to be examined three times or more to detect light infections.

The overall prevalence of parasitic infections recorded in faecal samples of dogs collected from the environment and from the rectum of dogs in the present study is low when compared to the 98.36% - 100% gastrointestinal helminths recovered from dogs in

Ethiopia during necropsies (Samuel 1990 and Shimelis 1994). It has been suggested that a higher prevalence of infection is usually recorded in necropsies compared to the faecal examination of dogs. This has been indicated by Doganay and Oge (1993) who reported a prevalence of 52.19% in faecal samples of stray dogs and 69.23 % in the same dogs after necropsy. According to Verster (1979), the most reliable method of determining the prevalence of gastrointestinal helminths is the recovery at necropsy.

Age of dogs and parasites in dog faecal samples from the rectum

In the present study similar infection rates were observed in the rectal samples of young and adult dogs. It has, however, been reported that young dogs shed helminths eggs more frequently than adults (Overgaauw 1997b; Oliveira-Sequeira, Amarante, Ferrari and Nunes 2002). Since most of the examined dogs were free-roaming and untreated, they were infected with a wide range of parasites and both young and adult dogs may contribute significantly to the contamination of the environment with eggs of parasites.

Cat faecal samples

As there are no epidemiological data on the prevalence of intestinal parasites of cats in Ethiopia, comparison is possible only to surveys conducted in other countries. In the present study 66.23% of cat faecal samples were positive. The prevalence is high when compared to the reported prevalence of 2% in faecal samples of cats in breeding colonies, examined using a $ZnSO_4$ sedimentation-floatation technique in the Netherlands (Overgaauw and Boersema 1998a). According to Overgaauw and Boersema (1998a), the low prevalence of nematode infections in cats was due to the absence of risk factors in the breeding catteries, such as the absence of infected paratenic hosts, outdoor exposure

to helminth eggs and treatment and care given to the cats. High gastrointestinal helminths loads with a prevalence of 65% in South Africa (Baker *et al*. 1989) and 89.7% in Spain (Calvete *et al*. 1998) were reported from necropsies of stray cats. As indicated before, higher prevalences are usually reported from the examination of animals after necropsy compared to the examination of faecal samples and from stray cats compared to those that are better cared for.

Soil samples

The overall soil contamination rate recorded in the present study (33.88%) for helminthic parasites in soil samples collected from the environment is in agreement with those of comparable studies that reported a prevalence of 35.3% to 36.5% in Nepal (Rai, Uga, Ono, Rai and Matsumura 2000) and 38% in gardens in Ireland (Holland, O'Connor, Taylor, Hughes, Girdwood and Smith 1991). This figures are higher than the reported prevalence of 1.24% found in soil samples from public parks examined using the sucrose floatation and centrifugation technique after washing and sieving in eastern Spain (Ruiz de Ybanez *et al*. 2001). The low prevalence in the public parks was attributed to the low numbers of stray dogs and cats, absence of sandpits for cats to deposit faeces, and soil type of the sampling areas in Spain. According to Mizgajska (2001), factors that could influence the result of soil examination are sample site selection, number and volume of samples, depth of sampling, season of examination, method of egg recovery, soil type, preservation of samples and laboratory skills.

***Toxocara canis* (Werner, 1782)**

In the present study *T. canis* eggs were recovered from 20.18% of faecal deposits of dogs collected from the environment. Comparable results (25.5%) were reported for dog faecal deposits collected from roadsides and public places in Egypt (Abou-Eisha and Abdel-Aal 1995). They used a combination of sedimentation and floatation techniques for faecal examination. The prevalence in the present study was, however, high compared to the 3.6% for faecal samples of an owned dog population, examined using a saturated sugar floatation technique in Ireland (O'Sullivan 1997). Prevalences of *T. canis* eggs, using a $ZnSO_4$ floatation technique were 1.7% in faecal samples of dogs in Australia (Bugg *et al.* 1999), 2.9% in dog faecal samples collected from households (Overgaauw 1997a) and 8% - 15% in dog faecal samples collected in dog breeding kennels (Overgaauw and Boersema 1998b) in the Netherlands. The low prevalence of *T. canis* was attributed to the awareness of most of the pet owners about zoonotic infections, resulting in regular treatment of domestic pets, and the absence or rare occurrence of stray dogs. The relatively high prevalence in the dog breeding kennels was related to infection pressure in a closed environment. However, single faecal specimen examination for *T. canis* infection results in the underestimation of the prevalence by 20 to 35% in lightly infected dogs (Barriga 1991; O`Sullivan 1997).

The 30.87% *T. canis* infection rate recorded in faecal samples collected from the rectum of dogs in the present study was comparable to the 19% based on peri-anal adhesive tape swabs and rectal faecal samples of dogs in South Africa (Minnaar *et al*. 1999), and the 21.9% recorded in faecal samples from the rectum of stray dogs in Ankara, Turkey

(Doganay and Oge 1993), and the 31.5% based on faecal samples of dogs collected by owners of pets or taken directly from the rectum during examination at a veterinary clinic in Poland (Luty 2001). According to Surgan *et al*. (1980), faecal examination may, however, reveal as few as one-half of the *Toxocara* infections diagnosed during necropsy.

Toxocara canis prevalences of 52.46% (Shimelis 1994) and 83% (Samuel 1990) reported in Ethiopia were based on necropsies of dogs. The infection rates of 32.5% - 56.6% in Nigeria (Arene 1984; Umeche and Hogan 1989) and 82.6% in Ireland (O'Lorcain 1994) were reported from necropsies of dogs. The highest prevalence of *T. canis* was reported in unwanted, stray, sick and/or untreated dogs or those brought to the veterinary clinic for medical examination (Umeche and Hogan 1989; O'Lorcain 1994).

Usually intestinal parasites are more prevalent in younger dogs than in adults (Oliveira-Sequeira *et al*. 2002). This was observed in the present study for *T. canis* infections in dogs from which the age was known as faecal samples were collected from the rectum. The prevalence of *T. canis* was significantly higher in young (43.45%) compared to adult (9.45%) dogs. The high prevalence of *T. canis* in young dogs might be associated with the transplacental route of transmission from infected bitches to the fetuses (Furth, El-On and Hoida 1990; Luty 2001; Oliveira-Sequeira *et al*. 2002) and the lower resistance of young dogs against *T. canis* infections (Baker *et al*. 1989). Almost all the puppies of an infected bitch can be infected through transplacental transmission (Kassai 1999, Roberts and Janovy 2000).

A significantly higher prevalence of *T. canis* was observed in female dogs (44.12%) compared to male dogs (20.31%). A similar finding was reported in a study conducted by Doganay and Oge (1993). Nakauchi, Ohtaka, Morishima and Hiki (1993), Luty (2001) and Oliveira-Sequeira *et al*. (2002), however, reported a higher prevalence of *T. canis* infections in male than in female dogs. The significantly higher prevalence rates for *T. canis* in female (44%) compared to in male dogs in the present study might have been as a result of the examination of large numbers of lactating bitches that can acquire patent infections from the reactivation of larvae long surviving from a prenatally acquired infection, or as a result of ingesting larval stages in the faeces of their puppies (Sprent and Barrett 1964; Kassai 1999).

The species identity of eggs from soil samples could not be determined due to the morphological similarities of *T. canis* and *T. cati* eggs (Uga *et al*. 1996). According to Slifko, Smith and Rose (2000), the presence of *T. canis* eggs in soil can be used to indicate the potential for environmental contamination. The overall prevalence of *Toxocara* spp. eggs was 14.05%, varying from 8.33% (open fields) to 58.33% (dust from doghouses). Eggs of *Toxocara* spp. were reported with a prevalence of 24.4 % in soil samples from public places using a $ZnSO_4$ centrifugal floatation method in Britain (Borg and Woodruff 1973), 35-53% in soils from city backyards using a saturated $NaNO_3$ floatation method in Poland (Mizgajska 2001), 46% in soil from public parks and recreational areas using a $ZnSO_4$ floatation technique in a Caribbean community (Thompson *et al*. 1986), and 63.3% in sandpits collected from various areas using saturated $NaNO_3$ floatation and sedimentation technique in Japan (Shimizu 1993).

According to Mizgajska (2001), factors that affect the outcome of soil examinations are sample site selection, number and volume of samples, depth of sampling, season of examination, method of egg recovery, type of soil examined, preservation of samples and laboratory skills. The presence of a low prevalence of *Toxocara* spp. eggs in soil samples does, however, not mean that there is no risk of human infection. The high fecundity of female worms and the resistance and long period of survival of eggs in the environment, contribute to the potential danger of human infections (Jordan *et al*. 1993, Oliveira-Sequeira *et al*. 2002), especially in recreational areas and playgrounds, and particularly to children.

***Toxocara cati* (Schrank, 1788)**

The overall prevalence of *T. cati* in cat faecal samples collected from households and residential areas in this study (51.95%), was higher compared to the 8% in faecal samples of cats collected from catteries (Overgaauw and Boersema 1998a) and 21% in those of stray cats (Overgaauw 1997a) in the Netherlands. According to these authors, the low prevalence of infection was related to the absence of risk factors because of practices such as the fencing of outdoor areas, cleaning of the environment, limited access to the outdoor environment and to paratenic hosts, and the deworming of cats. A higher prevalence of *T.* cati is more frequently reported in stray cats than in household cats and in faecal samples of free-ranging farm cats (91%) in the United Kingdom (Yamaguchi *et al*. 1996) and in rectal faecal samples and intestinal contents obtained during the necropsy of feral cats (84%) in southern Tasmania (Milstein and Goldsmid 1997). Prevalences

reported during necropsy were 11% in South Africa (Baker *et al*. 1989), 42% in Ireland (O'Lorcain 1994) and 55.2% in Spain (Calvete *et al*. 1998), and most of these cats were unwanted, stray or sick.

A high prevalence of *T. cati* in an area may contribute considerably to the contamination of the environment. Since children have the habit of pica (Overgaauw 1997) and because eggs of *Toxocara* species are remarkably resistant and survive for long periods in the environment (Dolinsky, Burright and Donovick 1981; Jordan *et al*. 1993), the presence of *Toxocara* spp. eggs in the present study area undoubtedly poses a potential risk for human infection, especially in young children.

***Toxascaris leonina* (V. Linstow 1902)**

Toxascaris leonina was found in 3.44% of faecal deposits of dogs in the present study. Using the same diagnostic technique O'Sullivan (1997), reported a 7.1% infection of faecal samples of owned dogs in Ireland. Using a combination of sedimentation and floatation techniques, Abou-Eisha and Abdel-Aal (1995) reported a 32.1% infection of dog faecal deposits from roadsides and public places in Egypt. A low prevalence (0.4 – 1%) of *T. leonina* infection was reported in dog faecal samples from dog breeding kennels (Overgaauw and Boersema 1998b) and in dog faecal samples collected from households (Overgaauw 1997a) in the Netherlands. However, a much higher prevalence (30.46%) was reported in dog breeding colonies in Ireland (Fisher *et al*. 2002).

In the present study, a prevalence of 5.19% *T. leonina* was found in cat faecal samples. Previous studies in Africa or in Ethiopia didn't report the presence of *T. leonina* in cats. Studies conducted in other countries, however, reported 0.2% in faecal samples of domestic cats in London (Nichol *et al*. 1981b), 1.1% in faecal samples of feral cats in England (Nichol *et al*. 1981a) and 5.4% in faecal samples of stray cats in the Netherlands (Overgaauw 1997a). A higher prevalence (82%) was reported in faecal samples of free-ranging farm cats in the United Kingdom (Yamaguchi *et al*. 1996). This high prevalence of *T. leonina* in stray cats is probably related to the cats preying on infected wild rodents. In South Africa, *T. leonina* has never been reported from cats (Prof. Boomker, J. 2002, Prof. Dawie, J. Kok, 2003, personal communication). In the present study, *T. leonina* eggs were recorded in 4.13% of soil samples collected from households and residential areas. This prevalence is comparable to 4.1% recorded in samples from public parks in Turkey (Oge and Oge 2000). The contamination of the environment with the eggs of *T. leonina* may be a potential source of infection to domestic pets and rodents in the areas.

Hookworms

Because the eggs of different species of hookworms are closely similar in morphological appearance (Minnaar *et al*. 1999), species identification was not done in the present study. Hookworm eggs were found with a prevalence of 36.24% in dog faecal deposits and 38.7% in dog faecal samples taken from the rectum. Studies conducted in other countries showed prevalences of 1.3% in dog faecal droppings in Egypt (Abou-Eisha and Abdel-Aal 1995) and 17.4% in dog faecal samples collected from public parks and playgrounds in Zimbabwe (Mukaratirwa and Taruvinga 1999). Minnaar *et al*. (1999)

reported a higher prevalence of hookworms (90 – 93%) in dog faecal samples collected per rectum in South Africa. An infection rate of 58% was reported during necropsies of dogs in Ethiopia (Samuel 1990). The prevalence of hookworms in an area is related to the climatic conditions (high rainfall and warm temperatures) favouring the survival of the larvae in the eggs for a long period of time (Jenkins and Andrew 1993).

The prevalence of hookworm eggs in samples of cat faeces (19.48%) from household and residential areas is comparable to the 29.3% *Ancylostoma caninum* (Ercolani, 1859) reported during necropsies of stray cats in Spain (Calvete *et al*. 1998). Baker *et al*. (1989) reported 1.4% - 41% hookworm infections during necropsies of cats in the Pretoria area of South Africa. The prevalence of soil contamination with hookworm eggs (9.5%) in the present study was lower compared to the 11.8% in nursery schools, 19.4% in households, 36% in public parks and 100% in playgrounds reported in Argentina (Alonso *et al*. 2001). The variation in prevalence of hookworms was attributed to soil type (Abou-Eisha and Abdel-Aal 1995), geographical factors, social standing of residents in the sample areas and sample size (Minnaar *et al*. 1999). Hookworms have seasonal variations and are considered to be prevalent during the summer and autumn (Oliveira-Sequeira *et al*. 2002). The abundance of hookworms in the study area may pose significant health problems to the public, as hookworms of domestic pets can cause dermatitis (Oliveira-Sequeira *et al*. 2002) or cutaneous larva migrans in humans (Robinson *et al*. 1989, Jenkins and Andrew 1993 and Minnaar *et al*. 1999).

***Strongyloides* Grassi, 1879 spp.**

Strongyloides spp. were not reported in dogs by previous researchers in Ethiopia. The prevalence of *Strongyloides* species recovered from faecal samples collected from the rectum (2.17%) was slightly higher than that faecal droppings (1.15%). The low prevalence rates might be related to the diagnostic method (sugar floatation) used for faecal examination, and the day-to-day variability of larvae in the faeces (Roberts and Janovy 2000). Baermann's method is usually used for the detection of *Strongyloides stercoralis* larvae (Magnaval 1995 and Kassai 1999). Umeche and Hogan (1989) reported a 26.2% infection rate of *Strongyloides stercoralis* in dog faecal samples brought to veterinary clinics for examination in Nigeria and suggested that dogs constitute one of the sources of human infections with *Strongyloides stercoralis*. Robinson *et al*. (1989) reported a prevalence of 6% *Strongyloides* sp. in faecal deposits of well-cared-for dogs in Jamaica. Georgi and Sprinkle (1974) suggested that transmission of *S. stercoralis* from canines to humans could be possible. Human strongyloidiasis, caused by *S. stercoralis* is common in the study area (Ali *et al*. 1999). This parasite can be lethal to human beings, particularly to immunocompromised individuals (Robinson *et al*. 1989).

***Ascaris* (Linnaeus, 1758) spp.**

Ascarid nematodes are rather host-specific and it is therefore unusual to find egg/larval/adult stages of *Ascaris* sp. in cats or dogs, *T. canis* in cats, *T. cati* in dogs, eggs, sub-adult or adult worms of *T. canis* or *T. cati* in man. However, various cases of non-specificity have been documented as follows: *Ascaris lumbricoides* eggs in dogs (Doganay and Oge 1993), *Ascaris* sp. eggs in dogs (Seah, Hucal and Law 1975), *T. canis*

in cats (Baker *et al*. 1989), *T. cati* in dogs (Doganay and Oge 1993), *T. cati* adult or sub adult worms in man (Von Reyn *et al*.1978 and Eberhard and Alfano 1998) and *T. canis* adult worms in man (Bisseru *et al*. 1966). Such infections were suggested to be the result of accidental infections from close contact. *Ascaris* spp. with prevalence rates of 3.91% in soil samples, 8.49% in faecal droppings and 9.09% in rectal faecal samples of dogs were recorded in the present study. Although, *Ascaris* spp. eggs or worms were not reported in faecal samples or necropsies of dogs in previous studies in Ethiopia, a similar result to the present study with a prevalence of 21.3% *Ascaris* spp. eggs in dogs was reported as the third most common helminth next to *Toxocara canis* and tapeworms in faecal samples of dogs in Canada (Seah *et al*. 1975). Furthermore, Doganay and Oge (1993) reported *A. lumbricoides* eggs in a dog but they couldn't recover adult worms during necropsy. It has been suggested by Seah *et al*. (1975) that the recovery of eggs of *Ascaris* spp. in faecal samples of dogs was due to a spurious rather than actual infection.

Ascaris lumbricoides is the most common parasite recorded in the human population in Ethiopia (Merid, Hegazy, Mekete and Teklemariam 2001). Surveys conducted in the present study area showed *A. lumbricoides* to be common in school children (Ali *et al*. 1999 and Haile *et al*. 1994) and the environment (Kefyalew 1998). Stray and free roaming dogs are known to practice coprophagia (Umeche and Hogan 1989; Jenkins and Andrew 1993) and thus may ingest human faeces from the environment. These eggs may have been passed directly through the gastrointestinal tract of the dogs without hatching and the ingestion of human excreta containing parasite eggs may cause spurious parasitism in dogs.

***Trichuris vulpis* (Frohlich, 1789)**

Trichuris vulpis was found in 3.44% of dog faecal deposits collected from the environment. This prevalence rate was comparable to the 4.8% in faecal samples of dogs in Brazil (Oliveira-Sequeira *et al*. 2002). The 3.19% prevalence found in samples of dog faeces collected from the rectum was also comparable to the 6% in dog rectal faecal samples and examined using a floatation method in a Faecalyzer® tube in South Africa (Minnaar and Krecek 2001). *Trichuris vulpis* was reported with prevalences of 5% from necropsies of dogs in Ireland (O'Sullivan 1997), 22.5 % (Umeche and Hogan 1989) and 56.7% (Arene 1984) in Nigeria. The low prevalence of *T. vulpis* in rectal faecal samples might be related to the fact that eggs are not continuously passed in the faeces or due to a low egg excretion (O'Sullivan 1997; Kassai 1999).

The *Trichuris spp.* contamination rate of 2.48% of soil samples in the environment was comparable to 1.8% of soil samples from public parks in Ankara, Turkey (Oge and Oge 2000), but was low compared to the 14% reported in soil samples taken from gardens of dog breeding kennels in The Netherlands (Overgaauw and Boersema 1998b). The kennels are different from other places and infection can be prevalent in these gardens.

Heavy infections with *Trichuris vulpis* may cause acute and chronic inflammation of the gastrointestinal tract of dogs (Soulsby 1982). It is much more difficult to find *T. vulpis* eggs using floatation techniques, especially in light infections (Prof. Dawie J. Kok, 2003, personal communication).

***Spirocerca lupi* (Rudolphi, 1809)**

In the present study, comparable prevalences of *S. lupi* were observed in dog faecal deposits in the environment (4.59%), in samples of dog faeces collected from the rectum (5.65%), and in soil samples (0.83%). Shimelis (1994) and Samuel (1990) reported prevalences of 32.79% and 33%, respectively, during necropsies of dogs in Ethiopia. According to Jordan *et al*. (1993) and Soulsby (1982), eggs of *S. lupi* cannot pass in the faeces or vomit unless the esophageal tumour is perforated into the lumen. As a result, *S. lupi* is usually reported with a low prevalence in faecal samples of dogs. In some cases, *S. lupi* was also reported with low prevalence rates during necropsies, e.g. 1.2% in dogs in South Africa (Verster 1979) and 1.9% in dogs in Brazil (Oliveira-Sequeira *et al*. 2002). Dogs get infected by the ingestion of either the intermediate host (dung beetles) or infected paratenic hosts (Kassai 1999; Roberts and Janovy 2000). According to Mylonakis, Koutinas, Liapi, Saridomichelakis and Rallis (2001), the life-style of dogs affects infection with *S. lupi* considerably. The parasite poses a considerable health problem in dogs being a cause of esophageal cancer (Roberts and Janovy 2000).

Cestodes

Since the eggs of the various *Taenia* Linnaeus, 1758 spp. cannot be distinguished microscopically from one another or from those of *Echinococcus* Rudolphi, 1801 spp. (Minnaar *et al*. 1999), the eggs were termed “taeniid” eggs in this study. The prevalence of tapeworm eggs was low in faecal samples of dogs in the environment (1.38%) and in faecal samples collected from the rectum (1.74%). Previous studies in Ethiopia reported high prevalence rates of *Taenia* spp. (50%) and *E. granulosus* (Batsch, 1786) (50%)

based on necropsies of dogs from Dire Dawa and East Haraghe (Samuel 1990), *E. granulosus* in stray dogs (33.33%) and bovine hydatidosis in slaughtered Zebu cattle (32.12%) in Mekelle (Yhidego 1997), *Taenia hydatigena* Pallas, 1766 (19.67%) and *E. granulosus* (9.86%) in dogs in the Debre Zeit area (Shimelis 1994).

Taenia spp. were reported in Ireland (O'Sullivan 1997) with a prevalence of 3.6% in faecal samples of owned dogs examined using a sugar floatation technique and 11% in necropsies of stray dogs. A high prevalence rate of *E. granulosus* (85%) and *T. hydatigena* (43.3%) was reported in gut samples taken from the esophagus to the sigmoid colon of dogs obtained from local butchers in Nigeria (Arene 1984). According to Arene (1984), the dogs have easy access to infected offal, which is disposed of at the various local slaughtering centers in Nigeria. Tapeworm eggs are often contained in proglottids and are released only after the mature segment disintegrates (Robinson *et al*. 1989). This may explain the low prevalence of eggs in faecal samples.

It is quite important to examine dogs for the presence of *E. granulosus* because of the potential health risk to humans (Jenkins and Andrew 1993). Hydatid disease has been reported to be one of the major public health problems in the lower Omo River basin in Ethiopia (Mekuria 1985; Lindtjorn, Kiserud, and Roth 1982). In addition, hydatidosis in animals results in an enormous economic loss as a result of offal condemnation, carcass weight loss and death of the infected animals in Ethiopia (Samuel 1990, Shimelis 1994 and Yhidego 1997).

Conclusions

This study showed that there are significant numbers of parasites in domestic pets and in the environment that could be potential sources of zoonotic infection to human beings, particularly to children. According to Lynch *et al*. (1988) almost nothing is known of the prevalence of *Toxocara* infection in the tropics, where infections with a wide range of intestinal parasites is common or extremely intense. This is true in countries like Ethiopia where there is poor personal hygiene, poor environmental sanitation, large numbers of stray dogs that are not treated for parasitic infections, and no awareness of the public in general and pet owners in particular about the potential health hazards of zoonotic infections from the domestic pets. Although the main objective of this study was to investigate the presence and prevalence of ascarids in faecal and soil samples in domestic pets and in the environment, the occurrence of other zoonotic parasite species in the study area that might also have a great influence on public health, were also reported on.

Based on the study results, the following suggestions and recommendations are forwarded

1. Proper disposal of dog, cat and human faeces.
2. Improvement of personal and environmental sanitation.
3. Decreasing the number of stray dogs and cats.
4. Regular surveillance and treatment of infected domestic pets.
5. Prevention of fouling of the environment with dog and cat faeces, particularly in children’s playgrounds.

6. Increasing the awareness of the public, particularly pet owners, about the potential risks of zoonoses from domestic pets.

These measures will contribute immensely towards the prevention and control of these parasites in the environment and in domestic animals and limit exposure of the population to these zoonotic parasites. In addition to this, it also indicates the necessity of the implementation of preventive and control measures by the responsible bodies and the communities at large. Further studies may be required to assess predisposing or risk factors, seroprevalence of toxocariasis in the human population, and a nationwide survey of the zoonotic parasites in domestic pets using a combination of diagnostic techniques, particularly for *T. canis*. Finally, such a survey may provide a baseline for further epidemiological studies in domestic pets and the environment.

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSIONS

Valuable information was gained in this study on the morphological aspects, modes of transmission and epidemiology of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina*. The present study revealed some additional morphological features. This includes size measurements of an adult female *T. canis* worm attaining a size of 27.5 cm and the presence of a precloacal median papilla in the male *T. canis* and *T. cati* worms. Though the presence of caudal alae was described in adult *Toxocara* spp. (Soulsby 1982), none of the examined worms were seen bearing caudal alae in the present study. Since spicules of male *T. leonina* were not protruding to the exterior through the cloaca in any of the adult male worms examined, no description of the structure of these organs could be given. Since most of the investigated larval stages were covered with a cuticlular sheath, details of external morphological structures were not examined under the scanning electron microscope.

This study has focused mainly on the external morphological aspects, but further studies of detailed morphological similarities and differences between the three species, using various techniques, are necessary. Besides this, it is quite important to conduct further studies on internal anatomical structures as well as DNA analysis in order to obtain a better understanding of these ascarids and to elucidate the question of strain variation between the *Toxascaris leonina* that infect dogs and cats, respectively. Since spurious ascarid infections in humans and *Ascaris* spp. in domestic pets were reported by previous researchers as well as in the present study, it may be important to undertake further

comparative morphological studies between ascarids of dogs and cats, as well as *Ascaris* spp. of humans and pigs. It is also possible that the eggs produced by human *A. lumbricoides* may pass through the gastrointestinal tract of dogs without hatching and further studies are also necessary to investigate this.

In the present study, transplacental transmission in *T. canis*, transmammary transmission in *T. canis* and *T. cati* and the absence of both transplacental and transmammary transmission in *T. leonina* were observed in mice infected with these parasites under laboratory conditions. However, transplacental transmission was observed only in mice infected during pregnancy. It may be necessary to carry out further studies under natural conditions and to investigate the mechanisms and factors that influence transplacental and transmammary transmission in one ascarid and inhibit it in others.

Though it has been considered that cats, particularly stray and wildcats, might get infected with *T. leonina* mainly through predating on infected paratenic hosts, this parasite was not able to develop to the adult stage from experimental infection of cats through ingesting third-stage larvae infected mice tissues. Besides this, the prevalence rates of *T. leonina* infection in cats were reported to be low compared to that in dogs in the literature reviewed. Though the presence of strain variations in *T. leonina* infecting dogs and cats has been described, investigation on this strain variation using modern techniques such as PCR and DNA analysis were not carried out. It is doubtful that cats usually acquire infections of *T. leonina* through predation of infected rodents and the adaptation of this parasite in cats is therefore questionable. Studies will have to be

conducted on *T. leonina* collected from dogs and cats as well as related canids and felines to provide more information on *T. leonina*.

The epidemiologies of ascarids of domestic pets were studied mainly in the developed world where there is proper management and care of domestic pets. Less attention was given to these parasites in the developing and third world countries like Ethiopia where socioeconomic burden exists and where health problems prevail. In most countries of Africa, dogs are usually unconstrained and are not treated for parasitic infections. The prevalence and zoonotic potential of these parasites originating from domestic pets is now known. In addition, the role of *Toxocara* spp. as a cause of visceral larva migrans, ocular larva migrans and covert toxocariasis in the human population, is unknown. The presence of a large number of unwanted and stray dogs and cats may significantly contribute to the contamination of the environment, mainly playgrounds and residential areas, with viable eggs of ascarids which in turn may play a large role in children acquiring infections. Epidemiological studies on the prevalence of toxocariasis in the human population and in domestic pets have to be conducted at a national level to lay the ground for proper prevention and control measures.

These parasites have a high reproductive potential, producing large numbers of eggs, which survive in the soil for prolonged periods of time, they have very successful modes of transmission (transplacental and transmammary transmission) and can infect a wide range of animals as paratenic hosts. It is therefore clear that considerable attention should be given to these parasites in the developing and poor countries.

CHAPTER VII

REFERENCES

ABE, N. & YASUKAWA, A. 1997. Prevalence of *Toxocara* spp. eggs in sandpits of parks in Osaka city, Japan, with notes on the prevention of egg contamination by fence construction. *Journal of Veterinary Medical Sciences*, **59**: 79-80.

ABOU-EISHA, A.M. & ABDEL-AAL, A.A. 1995. Prevalence of some zoonotic parasites in dog faecal deposits in Ismaili City. *Assiut Veterinary Medical Journal*, **33**: 119-125.

#ACHENEF, M., MARKOS, T., FESEHA, G., HIBRET, A. & TEMBELY, S. 1999. Coenurus cerebralis infection in Ethiopian highland sheep: Incidence and observations on pathogenesis and clinical signs. *Tropical Animal Health and Production*, **31**: 15-24.

#AGUDELO, C., VILLAREAL, E., CACERES, E., LOPEZ, C., ELJACH, J., RAMIREZ, N., HERNANDEZ, C. & CORREDOR, A. 1990. Human and dogs *Toxocara canis* infection in a poor neighbourhood in Bogota. *Memorias do Instituto Oswaldo Cruz*, **85**: 75-78.

ALDAWEK, A.M, LEVKUT, M., REVAJOVA, V., KOLODZIEYSKI, L., SEVEIKOVA, Z. & DUBINSKY, P. 2002. Larval toxocariasis in sheep: the immunohistochemical characterization of lesions in some affected organs. *Veterinary Parasitology*, **105**: 207-214.

ALI, I., MEKETE, G. & WODAJO, N. 1999. Intestinal parasitism and related risk factors among students of Asendabo Elementary and Junior Secondary School. Southwestern Ethiopia. *Ethiopian Journal of Health Development*, **13**: 157-161.

#ALONSO, J.M., STEIN, M., CHAMORRO, M.C. & BOJANICH, M.V. 2001. Contamination of soils with eggs of Toxocara in a subtropical city in Argentina. *Journal of Helminthology*, **75**: 165-168.

ARENE, F.I.O. 1984. Prevalence of toxocariasis and echinococcosis among dogs in the Niger Delta. *Journal of Tropical Medicine and Hygiene*, **87**: 207-209.

ARPINO, C., GATTINARA, G.C., PIERGILI, D. & CURATOLO, P. 1990. *Toxocara* infection and epilepsy in children: a case-control study. *Epilepsia*, **31**: 33-36.

BAKER, M.K., LANGE, L., VERSTER, A. & VAN DER PLAAT, S. 1989. A survey of helminths in domestic cats in the Pretoria area of Transvaal, Republic of South Africa. Part 1: The prevalence and comparison of burdens of helminths in adult and juvenile cats. *Journal of the South African Veterinary Association*, **60**: 139-142.

BARRIGA, O.O. 1991. Rational control of canine toxocariasis by the veterinary practitioner. *Journal of the American Veterinary Medical Association*, **198**: 216-221.

BEAVER, P.C., JUNG, R.C. & CUPP, E.W. 1984. *Clinical Parasitology*. 9th edit. Philadelphia Lea and Febiger, 825pp.

BEAVER, P.C., SYNDER, C.H., CARRERA, G.M., DENT, J.H. & LAFFERTY, J.W. 1952. Chronic eosinophilia due to visceral larva migrans. *Pediatrics*, **9**: 7-19.

BISSERU, B., WOODRUFF, A.W. & HUTCHINSON, R.I. 1966. Infection with adult *Toxocara canis*. *British Medical Journal*, **1**: 1583-1584.

BLAND, M. 1987. *An introduction to medical statistics*. Oxford. Oxford University Press, 365pp.

BORG, O.A. & WOODRUFF, A.W. 1973. Prevalence of infective ova of *Toxocara* species in public places. *British Medical Journal,* **4**: 470-472.

BRUCKNER, D.A. 1985. Serologic and intradermal tests for parasitic infections. *Pediatric Clinics of North America,* **32**: 1063-1075.

BUGG, R.J., ROBERTSON, I.D., ELLIOT, A.D. & THOMPSON, R.C.A. 1999. Gastrointestinal parasites of urban dogs in Perth, Western Australia. *Veterinary Parasitology,* **157**: 295-301.

CALVETE C., LUCIENTES J., CASTILLO J.A., ESTRADA R., GRACIA M.J., PERIBANEZ M.A. & FERRER M. 1998. Gastrointestinal helminth parasites in stray cats from the mid-Ebro Valley, Spain. *Veterinary parasitology,* **75**: 233-240.

CHILDS, J.E. 1985. The prevalence of *Toxocara* species ova in backyards and gardens of Baltimore, Maryland. *American Journal of Public Health*, **75**: 1092-1093.

CIARMELA, M.L., MINVIELLE, M.C., LORI, G. & BASUALDO, J.A. 2002. Biological interaction between soil fungi and Toxocara canis eggs. *Veterinary Parasitology,* **103**: 251-257.

CLEMETT, R.S., WILLIAMSON, H.J. HIDAJAT, R.R.; ALLARDYCE, R.A. & STEWART, A.C. 1987. Ocular *Toxocara canis* infections: diagnosis by enzyme immunoassay. *Australian and New Zealand Journal of Ophthalmology,* **15**: 145-150.

COX, D.M. & HOLLAND, C.V. 1998. The relationship between numbers of larvae recovered from the brain of *Toxocara canis*-infected mice and social behaviour and anxiety in the host. *Parasitology,* **116**: 579-594.

DE SAVIGNY, D.H., VOLLER, A. & WOODRUFF, A.W. 1979. Toxocariasis: serological diagnosis by enzyme immunoassay. *Journal of Clinical Pathology,* **32**: 284-288.

DE SOUZA, E.C. & NAKASHIMA, Y. 1995. Diffuse unilateral subacute neuroretinitis. Report of transvitereal surgical removal of a subretinal nematode. ***Ophthalmology,*** **102**: 1183-1186.

DODGE, J.S. 1980. *Toxocara canis*: the risks of infections. *New Zealand Medical Journal*, **91**: 24-26.

DOGANAY, A. & OGE, S. 1993. The prevalence of ascariasis in stray dogs in Ankara. *Ankara Universitesi Veteriner Fakultesi Dergisi,* **40**: 552-562.

DOLINSKY, Z.S., BURRIGHT, R.G. & DONOVICK, P.J. 1981. Behavioral effects of lead and *Toxocara canis* in mice. *Science,* **213**: 1142-1144.

DUBEY, J.P. 1969. Migration and development of *Toxascaris leonina* larvae in mice. *Tropical and Geographical Medicine,* **21**: 214-218.

DUBINSKY, P., AKAO, N., REITEROVA, K. & KONAKOVA, G. 2000. Comparison of the sensitive screening kit with two ELISA sets for detection of anti-Toxocara antibodies. *Southeast Asian Journal of Tropical Medicine and Public Health,* **31**: 394-398.

DUBINSKY P., HAVASIOVA-REITEROVA, K., PETKO, B., HOVORKA I. & TOMASOVICOVA, O. 1995. Role of small mammals in the epidemiology of toxocariasis. *Parasitology,* **110**: 187-193.

DUNN, A.M. 1978. *Veterinary Helminthology*. 2nd edition. William Heinemann Medical Books Limited, London, UK, 323pp.

DUNSMORE, J.D., THOMPSON, R.C. & BATES, I.A. 1984. Prevalence and survival of *Toxocara canis* eggs in the urban environment of Perth, Australia. ***Veterinary Parasitology,*** **16**: 303-311.

DZBENSKI, T.H., BITKOWSKA, B. & GOLAB, E. 1999. Detection of a circulating antigen of *Toxocara canis* in the sera of naturally infected men and experimental animals. *Acta Parasitologica,* **44**: 137-141.

EBERHARD, M.L. & ALFANO, E. 1998. Adult *Toxocara cati* infections in U.S. Children: Report of four cases. *American Journal of Tropical Medicine and Hygiene,* **59**: 404-406.

#ESPANA, A., SERNA, M.J., RUBIO, M., REDONDO, P. & QUINTANILLA, E. 1993. Secondary urticaria due to toxocariasis: possibly caused by ingesting raw cattle meat? *Journal of Investigational Allergology and Clinical Immunology*, **3**: 51-52.

FEKADU, M. 1982. Rabies in Ethiopia. *American Journal of Epidemiology,* **115**: 266-273.

#FENOY, S., CUELLAR, C. & GUILLEN, J.L. 1996 Seroprevalence of toxocariasis in children and adults in Madrid and Tenerife, Spain. *Journal of Helminthology,* **70**: 109-113.

FISHER, M.A., MURPHY, M.G. & SIEDEK, E.M. 2002. Epidemiology of *Toxascaris leonina* infection post-weaning within a colony of dogs. *Journal of Helminthology*, **76**: 27-29.

FURTH, M., EL-ON, J. & HOIDA, G. 1990. Prevalence of helminths in dogs in the Hadera district of Israel. *Israel Journal of Medical Sciences,* **26**: 636-637.

GAMATCHU, D. 1977. Aspects of climate and water budget in Ethiopia. Addis Ababa University Press. Addis Ababa.

GARCIA, L. S. & ASH, L.R. 1979. *Diagnostic parasitology clinical laboratory manual*. Mosby Company. London, 174pp.

#GEORGI, J.R. & SPRINKLE, C.L. 1974. A case of human strongyloidosis apparently contracted from asymptomatic colony dogs. *American Journal of Tropical Medicine and Hygiene,* **23**: 899-901

GILLESPIE, S.H. 1993. Human toxocariasis. Communicable disease report. *Communicable Disease Report Review,* **3**: 140-143.

GILLESPIE, S.H., PEREIRA, M. & RAMSEY, A. 1991. The prevalence of *Toxocara canis* ova in the soil samples from parks and gardens in the London area. *Public Health,* **105**: 335-339.

GLICKMAN, L.T. & SCHANTZ, P.M. 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiologic Reviews,* **3**: 230-250.

GROVE, I. D. 1990**.** *A History of Human Helminthology*. CAB International, Wallingford, 848pp.

GUTERBOCK, W.M. & LEVINE, N.D. 1977. Coccidia and intestinal nematodes of East Central Illinois cats. *Journal of the American Veterinary Medical Association,* **170**: 1411-1413.

HAILE, G., JIRA, C. & MOLA, T. 1994. Intestinal parasitism among jiren elementary and junior secondary school students in Southwestern Ethiopia. *Ethiopian Journal of Health Development,* **8**: 37-41.

#HASSAN, A.T. & EL-MANAWATY, N.H. 1994. Experimental murine toxocariasis histopathological study of chronic renal infection, transplacental transmission and ultrastractural study of eggshell. *Journal of the Egyptian Society of Parasitology,* **24**: 333-339.

HERRMANN, N., GLICKMAN, L.T, SCHANTZ, P.M., WESTON, M.G. & DOMANSKI, L.M. 1985. Seroprevalence of zoonotic toxocariasis in the United States: 1971-1973. *American Journal of Epidemiology,* **122**: 890-896.

HOLLAND, C.V. & COX, D.M. 2001. *Toxocara* in the mouse: a model for parasite-altered host behaviour? *Journal of Helminthology,* **75**: 125-135.

HOLLAND, C., O'CONNOR, P., TAYLOR, M.R., HUGHES, G. GIRDWOOD, R.W. & SMITH, H. 1991. Families, parks, gardens and toxocariasis. *Scandinavian Journal of Infectious Diseases,* **23**: 225-231.

HOLLAND, C.V., O'LORCAIN, P., TAYLOR, M.R. & KELLY, A. 1995. Sero-epidemiology of toxocariasis in school children. *Parasitology,* **110**: 535-545.

HUMBERT, P., NIEZBORALA, M., SALEMBIER, R., AUBIN, F., PIARROUX, R., BUCHET, S. & BARALE, T. 2000. Skin manifestations associated with toxocariasis: a case-control study. *Dermatology,* **201**: 230-234.

JACOBS, D.E., ARAKAWA, A., COURTNEY, C.H., GEMMELL, M.A., MCCALL, J.W., MYERS, G.H. & VANPARIJS, O. 1994. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of anthelmintics for dogs and cats. *Veterinary Parasitology,* **52**: 179-202.

JASKOSKI, B.J., BARR, V. & BORGES, M. 1982. Intestinal parasites of well-cared-for dogs: An area revised. *American Journal of Tropical Medicine and Hygiene,* **31**: 1107-1110.

JENKINS, D.J. & ANDREW, P.L. 1993. Intestinal parasites in dogs from an aboriginal community in New South Wales. ***Australian Veterinary Journal,* 70**: 115-116**.**

JORDAN, H.E., MULLINS, S.T. & STEBBINS, M.E. 1993. Endoparasitism in dogs: 21,583 cases (1981-1990). *Journal of American Veterinary Medical Association,* **203**: 547-549.

#KARBACH, G. & STOYE, M. 1982. Occurrence of prenatal and galactogenic infections caused by *Toxascaris leonina* Leiper 1907 (Ascaridae) in the mouse. ***Journal of Veterinary Medicine,* 29**: 219-230.

KASSAI, T. 1999. *Veterinary Helminthology*. Butterworth-Heinemann, Oxford. 260pp.

KAYES, S.G. & OAKS, J.A. 1976. Effect of inoculum size and length of infection on he distribution of *Toxocara canis* larvae in the mouse. ***American Journal of Tropical Medicine and Hygiene,* 25**: 573-580.

KEFYALEW, E. 1998. Soil contamination with *Ascaris lumbricoides* ova as an indicator of environmental hygiene in kindergartens and elementary schools of Jimma Town. *B.Sc. Thesis*. Jimma Institute of Health Sciences, School of Medical Laboratory Technology. Jimma, Ethiopia, 27pp.

KIELAR, R.A. 1983**. *Toxocara canis*** endophthalmitis with low ELISA titer. ***Annals of Ophthalmology,* 15**: 447-449.

KINCEKOVA, J., DUBINSKY, P. & PIROCHOVA, O. 1995. Transmission of anti-*Toxocara* antibodies from mothers to neonates. *Helminthologia*, **32**: 175-179.

KIRKPATRICK, C.E. 1988. Epidemiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital. *Veterinary Parasitology,* **30**: 113-124.

#KNAUS, B.U. & FEHLER, K. 1989. *Toxoplasma gondii*-Infektionen und Oozystenausscheidung bei Hauskatzen und ihre Bedeutung fur die Epidemiologie und Epizootiologie der Toxoplasmose. [*Toxoplasma gondii* infections and oocyst shedding in domestic cats and the significance of this for the epidemiology and epizootiology of toxoplasmosis]. *Angewandte Parasitologie,* **30**: 155-60.

LEE, K.T., MIN, H.K. & **SOH, C.T.** 1976. Transplacental migration of *T. canis* larvae in experimentally infected mice. *Journal of Parasitology,* **62**: 460-465.

***LEIPER, R.**T. 1907. Two new genera of nematodes occasionally parasitic in man. *British Medical Journal*, **2422:** 1996-1298.

LIN, D.S., LAI, S.S., BOWMAN, D.D., JACOBSON, R.H., BARR, M.C. & GIOVENGO, S.L. 1990. Feline immunodeficiency virus, feline leukaemia virus, *Toxoplasma gondii*, and intestinal parasitic infections in Taiwanese cats. *British Veterinary Journal*, **146**: 468-475.

LINDTJORN, B., KISERUD, T. & ROTH, K. 1982. Hydatid disease in some areas of southern Ethiopia. *Ethiopian Medical Journal*, **20**: 185-188.

LUTY, T. 2001. Prevalence of species of *Toxocar*a in dogs, cats and red foxes from the Poznan region, Poland. *Journal of Helminthology*, **75**: 153-154.

LYNCH, N.R., EDDY, K., HODGEN, A.N., LOPEZ, R.I. & TURNER, K.J. 1988. Seroprevalence of *Toxocara canis* infection in tropical Venezuela. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, **82**: 275-281.

MAETZ, H.M., KLEINSTEIN, R.N., FEDERICO, D. & WAYNE, J. 1987. Estimated prevalence of Toxoplasmosis and Toxocariasis in Alabama. *Journal of Infectious Diseases,* **156**: 414.

MAGNAVAL, J.F. 1995. Comparative efficacy of diethylcarbamazine and mebendazole for the treatment of human toxocariasis. *Parasitology,* **110**:529-533.

MAGNAVAL, J.F., MICHAULT, A., CALON, N. & CHARLET, J.P. 1994. Epidemiology of human toxocariasis in La Reunion. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **88**: 531-533.

MEKURIA, T. 1985. Human hydatidosis in Ethiopia. *Ethiopian Medical Journal,* **23**: 81-87.

MERID, Y., HEGAZY, M., MEKETE, G. & TEKLEMARIAM, S. 2001. Intestinal helminthic infection among children at Lake Awassa area, South Ethiopia. *Ethiopian Journal of Health Development,* **15**: 31-38.

#MERSIE, A. 1993. Survey of echinococcosis in eastern Ethiopia. *Veterinary Parasitology,* **47**: 161-163.

MILSTEIN, T.C. & GOLDSMID, J.M. 1997. Parasites of feral cats from southern Tasmania and their potential significance. *Australian Veterinary Journal,* **75**: 218-219.

MINNAAR, W.N. & KRECEK, R.C. 2001. Helminths in dogs belonging to people in a resource-limited urban community in Gayteng, South Africa.

MINNAAR, W.N. KRECEK, R.C. & FOURIE, LJ. 2002. Helminths in dogs from a peri-urban resource-limited community in Free State province. *South African Veterinary Association,* **107**: 343-349.

MINNAAR, W.N. KRECEK, R.C. & RAJPUT, J.I. 1999. Helminth parasites of dogs from two resource-limited communities in South Africa. *Journal of the South African Veterinary Association,* **70**: 92-94.

MIZGAJSKA, H. 2001. Eggs of *Toxocara* spp. in the environment and their public health implications. *Journal of Helminthology*, **75**: 147-151.

MORGAN, U.M. 2000. Detection and characterization of parasites causing emerging zoonoses. *International Journal for Parasitology*, **30**:1407-1421.

MUKARATIRWA, S. & TARUVINGA, M. 1999. A survey on environmental contamination of suburban parks and playgrounds in Harare, Zimbabwe, with canine helminths of zoonotic significance. *Journal of South African Veterinary Association,* **70**: 119-121.

MYLONAKIS, M.E., KOUTINAS, A.F., LIAPI, M.V., SARIDOMICHELAKIS, M.N. & RALLIS, T.S. 2001. A comparison of the prevalence of *Spirocerca lupi* in three groups of dogs with different life and hunting styles. *Journal of Helminthology,* **75**: 359-361.

***NAGAKURA, K., TCHIBANA, H. KANEDA, Y. & KATO, Y.** 1989. Toxocariasis possibly caused by ingesting raw chicken [letter]. *Journal of Infectious Diseases,* **160**: 735-736.

NAKAUCHI, K., OHTAKA, H., MORISHIMA, K. & HIKI, M. 1993. A survey on *Toxocara canis* of stray dogs in Ibaraki prefecture, Japan. *Japanese Journal of Parasitology,* **42**: 365-367.

NICHOLS, R.L. 1956. The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. *Journal of Parasitology*, **42**: 349-399.

NICHOL, S, BALL, S.J. & SNOW, K.R. 1981a. Prevalence of intestinal parasites in feral cats in some urban areas of England. *Veterinary Parasitology*, **9**: 107-110.

NICHOL, S., BALL, S.J. & SNOW, K.R. 1981b. Prevalence of intestinal parasites in domestic cats from the London area. *Veterinary Record,* **109**: 252-253.

Nunes, C.M., Tundisi, R.N., Heinemann, M.B., Ogassawara, S. & Richtzenhain, L.J. 1999. Toxocariasis: serological diagnosis by indirect antibody competition ELISA. *Revista Do Instituto De Medicina Tropical De Sao Paulo*. **41**: 95-100.

#OGE, S. & OGE, H. 2000. Prevalence of *Toxocara* spp. eggs in the soil of public parks in Ankara, Turkey. *Deutsche Tierarztliche Wochenschrift*, **107**: 72-75.

OKOSHI, S. & USUI, M. 1967a. Experimental studies on *Toxocara leonina*. I. Incidence of *T. leonina* among dogs and cats in Japan. *Japanese Journal of Veterinary science,* **29**: 185-194.

OKOSHI, S. & USUI, M. 1967b. Experimental studies on *Toxocara leonina*. II. Diagnosis and treatment of toxocariasis in dogs and cats. *Japanese Journal of Veterinary Science,* **29**: 245-250.

OKOSHI, S. & USUI, M. 1968. Experimental studies on *Toxascaris leonina.* V. Experimental infections of dogs and cats with eggs of canine, Feline and Felidae strains. *Japanese Journal of Veterinary Science,* **30**: 81-91.

OLIVEIRA-SEQUEIRA, T.C.G., AMARANTE, A.F.T., FERRARI, T.B. & NUNES, L.C. 2002. Prevalence of intestinal parasites in dogs in Sao Paulo State, Brazil. *Veterinary Parasitology,* **103**: 19-27.

O'LORCAIN, P. 1994. Epidemiology of *Toxocara* spp. in stray dogs and cats in Dublin, Ireland. *Journal of Helminthology*, **68**: 331-336.

OLSEN, O.W. 1974. *Animal parasites*. Their life cycle and ecology. 3rd edition. University Park Press. London, 562pp.

OSHIMA, T 1961a. Influence of pregnancy and lactation on migration of the larvae of *Toxocara canis* in mice. *Journal of Parasitology*, **47**: 657-660.

OSHIMA, T. 1961b. Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. *Journal of Parasitology*, **47**: 652-656.

O'SULLIVAN, E.N. 1997. Helminth infections in owned and stray dogs in Co. Cork, Ireland. *Irish Veterinary Journal Incorporating Irish Veterinary Times*, **50**: 108-110.

OVERGAAUW, P.A.M. 1997a. Prevalence of intestinal nematodes of dogs and cats in The Netherlands. *Veterinary quarterly*, **19**: 14-17.

OVERGAAUW, P.A.M. 1997b. Aspects of *Toxocara* epidemiology: Toxocarosis in dogs and cats. *Critical Reviews in Microbiology*, **23**: 233-251.

OVERGAAUW, P.A.M. & BOERSEMA, J.H. 1998a. A survey of *Toxocara* infections in cat breeding colonies in The Netherlands. *Veterinary Quarterly*, **20**: 9-11.

OVERGAAUW, P.A.M. & BOERSEMA, J.H. 1998b. Nematode infections in dog breeding kennels in The Netherlands, with special reference to *Toxocara*. *Veterinary Quarterly*, **20**: 12-15.

PAGE, A.P., RICHARDS, D.T., LEWIS, J.W., OMAR, H.M. & MAIZELS, R.M. 1991. Comparison of isolates and species of *Toxocara* and *Toxascaris* by

biosynthetic labelling of somatic and ES proteins from infective larvae. *Parasitology*, **103**: 451-464.

PAPADOPOULOS, H., HIMONAS, C., PAPAZAHARIADOU, M. & ANTONIADOU-SOTIRIADOU, K. 1997. Helminths of foxes and other wild carnivores from rural areas in Greece. *Journal of Helminthology*, **71**: 227-231.

PARSONS, J.C. 1987. *Ascarid* infection of cats and dogs. The veterinary clinics of North America. *Small Animal Practice*, **17**: 1307-1339.

POLLARD, Z.F., JARRETT, W.H., HAGLER, W.S., ALLAIN, D.S. & SCHANTZ, P.M. 1979. ELISA for diagnosis of ocular toxocariasis. *Ophthalmology*, **86**: 743-752.

#RADMAN, N.E., ARCHELLI, S.M., FONROUGE, R.D., DEL V GUARDIS, M., & LINZITTO, O. R. 2000. Human toxocarosis. Its seroprevalence in the city of La Plata. *Memorias do Instituto Oswaldo Cruz*, **95**: 281-285.

#RAI, S.K., UGA, S., ONO, K., RAI, G. & MATSUMURA, T. 2000. Contamination of soil with helminth parasite eggs in Nepal. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **31**: 388-393.

RAISTRICK, E.R. & HART, J.C. 1976. Ocular toxocariasis in adults. *British Journal of Ophthalmology*, **60**: 365-370.

READ, A.F. & SKORPING, A. 1995. The evolution of tissue migration by parasitic nematode larvae. *Parasitology*, **111**. 359-371.

REINECKE, R.K. 1983. *Veterinary Helminthology*. Durban: Butterworths, 392pp.

ROBERTS, L.S. & JANOVY, J.Jr. 2000. *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology*. 6th edition. McGraw-Hill. Singapore, 670pp.

ROBERTSON, I.D., IRWIN, P.J., LYMBERY, A.J. & THOMPSON, R.C.A. 2000. The role of companion animals in the emergence of parasitic zoonoses. International Journal for Parasitology, **30**: 1369-1377.

ROBINSON, R.D., THOMPSON, D.L. & LINDO, J.F. 1989. A survey of intestinal helminths of well-cared-for dogs in Jamaica, and their potential public health significance. *Journal of Helminthology,* **63**:32-38.

ROMEU, J., ROIG, J., BADA, J.L., RIERA, C. & MUNOZ, C. 1991. Adult human toxocariasis acquired by eating raw snails. *Journal of Infectious Diseases,* **164**: 438.

ROTH, R.M. & GLECKMAN, R.A. 1985. Human infections derived from dogs. *Postgraduate Medicine,* **77**: 169-73.

RUIZ DE YBANEZ, M.R., GARIJO, M.M. & ALONSO, F.D. 2001. Prevalence and viability of eggs of *Toxocara* spp. and *Toxascaris leonina* in public parks in eastern Spain. *Journal of Helminthology*, **75**: 169-173.

RUIZ DE YBANEZ, M.R., GARIJO, M., GOYENA, M. & ALONSO, F.D. 2000. Improved methods for recovering eggs of *Toxocara canis* from soil. *Journal of Helminthology*, **74**: 349-353.

#SADJJADI, S.M., KHOSRAVI, M., MEHRABANI, D. & ORYA, A. 2000. Seroprevalence of *Toxocara* infection in school children in Shiraz, southern Iran. *Journal of Tropical Pediatrics,* **46**: 327-330.

SAMUEL, T. 1990. External and GIT helminth parasites of dogs in Dire Dawa and East Hararghe. *DVM thesis*. Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia, 20pp.

SCHANTZ, P.M. & GLICKMAN, L.T. 1978. Current concepts in Parasitology. Toxocaral visceral larva migrans. *New England Journal of Medicine,* **298**: 436-439.

SCHANTZ, P.M. & GLICKMAN, L.T. 1979. Canine and human toxocariasis: the public health problem and the veterinarian's role in prevention. *Journal of the American Veterinary Medical Association,* **175**: 1270-1273.

SCHANTZ, P.M., MEYER, D. & GLICKMAN, L.T. 1978. Clinical, serologic and epidemiologic characteristics of ocular toxocariasis. *American Journal of Tropical Medicine and Hygiene,* **28**: 24-28.

SCHANTZ, P.M., WEIS, P.E., POLLARD, Z.F. & WHITE, M.C. 1980. Risk factors for Toxocaral ocular larva migrans: A case-control study. *American Journal of Public Health,* **70**: 1269-1272.

SCION IMAGE. 2000. Scion Corporation. National Institutes of Health (NIH). USA.

SEAH, S.K., HUCAL, G. & LAW, C. 1975. Dogs and intestinal parasites: a public health problem. *Canadian Medical Association Journal,* **112**: 1191-1194.

SEARL, S.S, MOAZED, K., ALBERT, D.M. & MARCUS, L.C. 1981. Ocular toxocariasis presenting as leukocoria in a patient with low ELISA titer to *Toxocara canis*. *Ophthalmology*, **88**: 1302-1306.

SHIMELIS, S. 1994. Study on canine gastrointestinal helminthiasis in Debre Zeit Region. *DVM thesis*. Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia, 36pp.

SHIMIZU, T. 1993. Prevalence of *Toxocara* eggs in sandpits in Tokushima City and its outskirts. *Journal of Veterinary Medical Sciences,* **55**: 807-811.

SHOOP, W.L. & CORKUM, K.C. 1987. Maternal transmission by *Alaria marcianae* (Trematoda) and the concept of amphiparatenesis. *The Journal of Parasitology,* **73**: 110-115.

SLIFKO, T.R., SMITH, H.V. & ROSE, J.B. 2000. Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology,* **30**: 1379-1393.

SLOVAK, A.J.M. 1984. Toxocaral antibodies in personnel occupationally concerned with dogs. *British Journal of Industrial Medicine,* **41**: 419.

SMITH, H.V. 1998. Detection of parasites in the environment. *Parasitology,* **117**: 113-141.

SOULSBY, E.J.L. 1982. *Helminths, arthropods* and *protozoa* of domesticated animals. Seventh edition. , Philadelphia: Lea and Febiger, 809pp.

SPRENT, J.F.A. 1952. On the migratory behaviour of the larvae of various Ascaris species in white mice. *Journal of Infectious Disease,* **90**: 165-176.

SPRENT, J.F.A. 1953. Intermediate hosts in *Ascaris* infection. *Journal of Parasitology*, **39**:38.

SPRENT, J.F.A. 1956. The life history and development of *Toxocara cati* (Schrank 1788) in domestic cat. *Parasitology*, **46**: 54-78.

SPRENT, J.F.A. 1958. Observation on the development of *Toxocara canis* (Werner, 1782) in the dog. *Parasitology,* **48**: 184-209.

SPRENT, J.F.A. 1959. The life history and development of *Toxascaris leonina* (von Linstow 1902) in the dog and cat, *Parasitology*, **49**: 330-371.

SPRENT, J.F.A. & BARRETT, G. 1964. Large roundworms of dogs and cats: Differentiation of *Toxocara canis* and *Toxascaris leonina. Australian Veterinary Journal,* **40**: 166-171.

***STURCHLER, D., WEISS, N. & GASSNER, M.** 1990. Transmission of toxocariasis [letter]. *Journal of Infectious Diseases*, **162**: 571

SURGAN, M.H., COLGAN, K.B., KENNETT, S.I. & PAFFMANN, J.V. 1980. A survey of canine toxocariasis and *Toxocara* soil contamination in Essex County, New Jersey. *American Journal of Public Health*, **70**: 1207-1208.

TAN, J.S. 1997. Human zoonotic infections transmitted by dogs and cats. *Archives of Internal Medicine*, **157**: 1933-1943.

TAYLOR, M.R., KEANE, C.T., O'CONNOR, P., MULVIHILL, E. & HOLLAND, C. 1988. The Expanded Spectrum of Toxocaral Disease. *Lancet*, **1**: 692-695.

TAYLOR, M.R., O'CONNOR, P., HINSON, A.R. & SMITH, H.V. 1996. *Toxocara* titres in maternal and cord blood. *Journal of Infection*, **32**: 231-233.

THOMPSON, D.E., BUNDY, D.A.P., COOPER, E.S. & SCHANTZ, P.M. 1986. Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. *Bulletin of the World Health Organization*, **64**: 283-290.

TOMASOVICOVA, O., HAVASIOVA-REITEROVA, K., DUBINSKY, P. & HOVORKA, I. 1993. Intrauterine and lactogenic transfer of *Toxocara canis* larvae in paratenic hosts. *Helminthologia*, **30**: 111-113.

UBELAKER, J.U. & ALLISON, V.F. 1975. Scanning electron microscopy of the eggs of *Ascaris lumbricoides*, *A. suum*, *T. canis*, and *T. mystax*. *Journal of Parasitology*, **61**:802- 807.

UGA, S. 1993. Prevalence of *Toxocara* eggs and number of faecal deposits from dogs and cats in sandpits of public parks in Japan. *Journal of Helminthology*, **67**: 78-82.

UGA, S., MATSUO, J., KIMURA, D., RAI, S.K., KOSHINO, Y. & IGARASHI, K. 2000. Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. *Veterinary Parasitology*, **92**: 287-294.

UGA, S., MINAMI, T. & NAGATA, K. 1996. Defecation habits of cats and dogs and contamination by *Toxocara* eggs in public park sandpits. *American Journal of Tropical Medicine and Hygiene*, **54**: 122-126.

UMECHE, N. & HOGAN, U.E.E. 1989. A study of intestinal helminths of dogs in Calabar, Nigeria. *Archivio Veterinario Italiano,* **40**: 128-131.

URQUHART, G.M., ARMOUR, J., DUNCAN, J.L. & JENNINGS, F.W. 1989. *Veterinary Parasitology*. Inc New York Churchill Livingstone, 286pp.

VERSTER, A. 1979. Gastro-intestinal helminths of domestic dogs in the Republic of South Africa. *Onderstepoort Journal of Veterinary Research*, **46**: 79-82.

VON REYN, C.F., ROBERTS, T.M., OWEN, R. & BEAVER, P.C. 1978. Infection of an infant with adult *Toxocara cati* (Nematoda). *The Journal of Pediatrics,* **93**: 247-249.

WANG, C., HUANG, C.Y., CHAN, P.H., PRESTON, P. & CHAU, P.Y. 1983. Transverse myelitis associated with larva migrans: Finding of larva in cerebrospinal fluid. *Lancet,* **1**: 423.

WILCOCKS, C. AND MANSON-BAHR, P.E.C. 1978. *Manson's Tropical Diseases*. 7th edition. Macmilan publishers, London. 1164pp.

WORLD HEALTH ORGANIZATION (WHO). 1967. Control of ascariasis. *Technical report series No*. 379.

WORLD HEALTH ORGANIZATION (WHO). 1998. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. *World Health Organization*. Geneva.

WORLEY, G, GREEN, J.A. FROTHINGHAM, T.E. STURNER, R.A. WALLS, K.W., PAKALNIS, V.A. & ELLIS, G.S. 1984. *Toxocara canis* infection: Clinical and epidemiological associations with seropositivity in Kindergarten children. *The Journal of Infectious Diseases,* **149**:591-597.

YAMAGUCHI, N., MACDONALD, D.W., PASSANISI, W.C., HARBOUR, D.A. & HOPPER, C.D. 1996. Parasite prevalence in free-ranging farm cats, Felis silvestris catus. *Epidemiology and infection,* **116**: 217-23.

YAMASAKI, H., ARAKI, K., LIM, P.K.C., ZASMY, N., MAK, J.W., TAIB, R. & AOKI, T. 2000. Development of a highly specific recombinant *Toxocara canis* second stage larva excretory antigen for immunodiagnosis of human toxocariasis. *Journal of Clinical Microbiology,* **38**: 1409-1413.

YHIDEGO, H. 1997. Hydatidosis/Echinococcosis: Prevalence and economic impact in bovine at Mekele Municipal abattoir, zoonosis and infection in dogs. *DVM thesis.* Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia, 34pp.

YOSHIDA, M., SHIRAO, Y., ASAI, H., NAGASE, H., NAKAMURA, H., OKAZAWA, T., KONDO, K., TAKAYANAGI, T.H., FUJITA, K. & AKOA, N. 1999. A retrospective study of ocular toxocariasis in Japan: Correlation with antibody prevalence and Ophthalmological findings of patients with uveitis. *Journal of Helminthology,* **73**:357-361.

ZHU, X.Q, GASSER, R.B., CHILTON, N.B. & JACOBS, D.E. 2001. Molecular approaches for studying ascaridoid nematodes with zoonotic potential, with an emphasis on *Toxocara* species. *Journal of Helminthology,* **75**:101-108.

***** Articles not seen in its original form.

Only Abstracts were seen

CHAPTER VIII

SUMMARY

The present study has provided detailed and comprehensive information on the morphological aspects, life cycle, and epidemiology of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina*. The overall results of the study results are briefly summarized as follows:

1. In the morphological studies of these parasites, it has been observed that there are gross morphological similarities in the developmental stages of *T. canis*, *T. cati* and *T. leonina*. However, closer and detailed observations have revealed that the various developmental stages differ from one another in their fine morphological features. The observed morphological differences were:

a. Eggs of *T. canis* and *T. cati* look very similar under the light microscope, in some eggs it seems that the surface pits and ridges of *T. canis* are larger and coarser than in the eggs of *T. cati*. However, unlike the eggs of *Toxocara* spp., eggs of *T. leonina* have no surface pits and ridges but are smoother on the surface.

b. Second-stage larvae of *T. canis* recovered from the eggs and from the infected tissues of mice were stumpy and vigorously motile. The larval stages from both sources were similar in their morphological features and had comparable size measurements indicating that larval stages did not undergo development

in the paratenic host. Like the larvae of *T. canis*, second-stage larva of *T. cati* recovered from the eggs and from the infected tissues of mice were stumpy and vigorously motile. The larval stages from both sources were similar in their morphological features and had comparable size measurements. The second-stage larvae of *T. canis* recovered from eggs or from tissues of mice had a greater width than the comparable second-stage larvae of *T. cati*. Unlike the larval stages recovered from the embryonated eggs of *T. leonina*, larvae recovered from infected tissues of mice were longer and wider and had more distinct morphological features. This indicated that larval stages recovered from the tissues of infected mice had probably undergone development to third-stage larvae.

c. Adults *T. canis*, *T. cati* and *T. leonina* resemble each other in their gross morphological features, but also differ in their detailed and fine structural features. The major differences were on the shape of cervical alae, the presence or absence of an esophageal ventriculus, the position of the vulva in the female worms, as well as the number and arrangement of papillae on the tail of the male worms.

d. Precloacal median papilla were recorded and described in males of *T. canis* and *T. cati*. Previous researchers did not describe the presence of these structures in male *Toxocara* spp. worms. In the present study, a female *T.*

canis worm with a length of 27.5 cm was recovered from an infected dog and it was larger than any recorded in the literature.

2. In the study of the modes of transmission of ascarids, it has been observed that transplacental transmission of *T. canis* occurred in mice infected during but not before pregnancy. Transmammary transmission of *T. canis* and *T. cati* larvae had also occurred in mice infected prior to or during pregnancy. However, unlike in *T. canis* and *T. cati*, had neither transplacental nor transmammary transmission occurred in *T. leonina*. Larval stages were recovered from most tissues and organs of infected mice, and the larvae gradually accumulated in various extraintestinal tissues for the three ascarid species and especially in the brain of mice infected with *T. canis*.

3. The study on *T. leonina* transmission to the definitive hosts through experimentally infected mice as paratenic host, has indicated that dogs can be infected by ingesting infected tissues of mice that were infected with *T. leonina* infective eggs originally obtained from dogs. However, in cats this was not possible and it has been suggested that cats may not be the proper definitive host for *T. leonina* originating from dogs.

4. In an epidemiological survey of *Toxocara* spp. *T. leonina*, and other intestinal helminths of dogs and cats in Jimma, Southwestern Ethiopia, it was shown that the overall prevalence rates for parasitic infection were 60.78% in faecal samples of dogs from the environment, 78.60% in faecal samples of dogs taken from the rectum, 66.23% in faecal samples of cats and 33.38% in soil samples were recorded. Parasite

species identified were *Toxocara canis*, *T. cati*, *Toxascaris leonina*, hookworms, *Spirocerca lupi*, *Trichuris vulpis*, taeniids, *Ascaris* species and *Strongyloides* species. A relatively high mean egg count of 15.25 was observed for *T. canis* in dog faecal droppings collected from the environment. The potential of these parasites as a cause of zoonotic infections, ways of prevention of infection and control measures were discussed.

HOOFSTUK IX

OPSOMMING

Die huidige studie verskaf omvattende inligting met betrekking tot aspekte van die morfologie, lewensiklus, en epidemiologie van *Toxocara canis*, *Toxocara cati* en *Toxascaris leonina*. Die resultate van die studie word kortliks as volg opgesom:

1. In die morfologiese studie van hierdie parasiete is waargeneem dat daar belangrike morfologiese ooreenkomste tussen die ontwikkelende stadiums van *T. canis*, *T. cati* en *T. leonina* bestaan. ‘n Deeglike en indiepte ondersoek het egter aangetoon dat die onderskeie stadiums op die fyner morfologiese vlak wel van mekaar verskil. Die waargenome verskille in morfologie was die volgende:

a. Eiers van *T. canis* en *T. cati* lyk baie dieselfde onder die ligmikroskoop, in sommige eiers blyk dit asof die oppervlakgaatjies en -riwwe van *T. canis* groter en growwer is as in die eiers van *T. cati*. In die geval van *T. leonina* besit die eiers egter geen sodanige oppervlakgaatjies en -riwwe nie en is die oppervlak in hierdie spesie gladder.

b. Tweede larwaal stadiums van *T. canis* wat uit eiers asook van geïnfekteerde muisweefsel verkry is, was kort, dik en baie beweeglik. Die larwes van beide bronne het ten opsigte van hul morfologiese eienskappe ooreengekom en het ook goed vergelyk in liggaamgroottes wat ‘n aanduiding is dat die larwes nie ontwikkeling in die parateniese gasheer ondergaan het nie. Net soos in die

geval van *T. canis*, was die tweede larwaalstadium van *T. cati* herwin uit eiers sowel as geïnfekteerde muisweefsel, ook kort, dik en baie beweeeglik. Die larwaalstadiums vanuit beide bronne het morfologies sowel as in hul liggaamsgroottes ooreengekom. Die tweede larwaalstadiums van *T. canis* vanuit eiers of muisweefsel was wyer as die ooreenstemmende tweede larwaalstadium van *T. cati*. Anders as die larwaalstadiums verkry uit geëmbrioneerde eiers van *T. leonina*, was die larwes herwin uit geïnfekteerde muisweefsel langer en wyer met onderskeidende morfologiese kenmerke. Hierdie verskille dui moontlik daarop dat die larwes vanuit geïnfekteerde muisweefsel moontlik ontwikkeling na die derde larwaalstadium ondergaan het.

c. Volwassenes van *T. canis, T. cati* en *T. leonina* toon baie ooreenkomste in hulle algemene morfologie, maar verskil ook op grond van hul fyner strukture. Die hoof verskille is in die vorm van die nekvleuels, die teenwoordigheid of afwesigheid van ‘n esofageale ventrikulus, die posisie van die vulva in die vroulike wurms, asook die getal en rangskikking van papillas op die stert van die manlike wurms.

d. Prekloakale mediane papillas is waargeneem en beskryf in die mannetjies van *T. canis* en *T. cati*. Hierdie strukture is nooit voorheen deur navorsers in mannetjies van *Toxocara* spp. beskryf nie. In die huidige studie is ‘n wyfie *T.*

canis van 27.5 cm in ‘n geïnfekteerde hond gevind, hierdie wurm was langer as enige waarnemings in die literatuur.

2. In die studie van die oordragingsmetodes by verteenwoordigers van die Ascariidae, is waargeneem dat transplasentale oordraging by *T. canis* in muise plaasgevind het wat tydens en nie voor swangerskap geïnfekteer is nie. Oordraging van larwes van *T. canis* and *T. cati* tydens die soogtyd het ook plaasgevind in muise wat voor of tydens swangerskap geïnfekteer is. Anders as in *T. canis* en *T. cati* het transplasentale oordraging en oordraging tydens die soogtyd glad nie in *T. leonina* voorgekom nie. Larwale stadiums is uit die meeste weefsel en organe van geïnfekteerde muise herwin, en die larwes van die drie spesies het geleidelik in die onderskeie ekstra-intestinale weefsel opgehoop, veral in die brein van die muise wat met *T. canis* geïnfekteer was.

3. Die studie van oordraging van *T. leonina* na die bepalende gasheer deur muise wat eksperimenteel geïnfekteer is, het aangedui dat honde wel geïnfekteer kan word deur muisweefsel in te neem wat besmet is met infektiewe *T. leonina* eiers wat oorspronklik van honde verkry is. In katte was dit egter nie moontlik nie en word voorgestel dat katte dalk nie die korrekte bepalende gasheer vir *T. leonina* is wat oorspronklik uit honde verkry is nie.

4. In ‘n epidemiologiese opname van *Toxocara* spp. *T. leonina*, en ander intestinale helminte van katte en honde in Jimma, suidwestelike Etiopië, is aangetoon dat die algemene persentasie besmetting vir parasitiese infeksies 60.78% in fekale

hondemonsters uit die omgewing was, 78.60% in fekale hondemonsters vanuit die rektum, 66.23% in fekale katmonsters en 33.38% in grondmonsters. Parasietspesies wat geïdentifiseer is, was die volgende; *Toxocara canis*, *T. cati*, *Toxascaris leonina*, haakwurms, *Spirocerca lupi*, *Trichuris vulpis*, verteenwoordigers van die Taeniidae, *Ascaris* spesies en *Strongyloides* spesies. ‘n Relatiewe hoë gemiddelde eiertelling van 15.25 is vir *T. canis* in hondemis waargeneem wat uit die omgewing versamel is. Die potensiaal van hierdie parasiete as ‘n oorsaak van soönotiese infeksies, maniere om infeksies te voorkom en beheermaatreëls is bespreek.

APPENDIX I

DEFINITION OF TERMS

Alae: Lateral wing-like expansions of the cuticle.

Amphid: Sensory organ on each side of the ‘head” of nematodes

Definitive host: Host in which a parasite achieves sexual maturity.

Denticles: Small toothlike projections.

Epidemiology: A study concerned with all ecological aspects of a disease to explain its transmission, distribution, prevalence and incidence.

Host: An organism that harbours a parasite.

Intensity: Number of parasites in an infected host, i.e. the infrapopulation.

Intermediate host: Host in which a parasite develops to some extent but not to sexual maturity and which is an essential link in the life cycle.

Mean intensity: The average number of parasites per infected host.

Moult: Periodic cuticle replacement during the life cycle of nematodes.

Operculum: Lid-like structure on the egg shell through which the larva of a parasite escapes.

Parasite: An organism that depends on another organism (the host) for shelter, transport and/or metabolites.

Paratenic host: A host in which a parasite survives without undergoing further development.

Patent: Stage in an infection at which infectious agents produce evidence of their presence for example the onset of egg production.

Phasmid: Sensory pit on each side near the end of the tail of nematodes of the subclass Rhabditia.

Prepatent: Developmental stage in an infection before agents produce evidence of their presence.

Prevalence: The number of individuals infected with a particular parasite species as a percentage of the number of hosts examined.

Reservoir: An infectious agent maintained in a host that can serve as a source of infection for humans or domestic animals.

Strain: Refers to a specific population of organisms which share, and are characterized by, particular biological or epidemiological features.

Spicule: Tiny, needle-like copulatory structures in nematodes.

Vulva: Opening of the uterus to the exterior in nematodes.

Zoonoses: Disease of animals that is transmissible to humans.

APPENDIX II. PERMITS

Permits from ethics committee for the study of

1. Transplacental and transmammary transmission modes of transmission of *Toxocara canis, Toxocara cati* and *Toxascaris leonina* in a paratenic host.
2. Epidemiology of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* in Jimma, southwestern Ethiopia (written in Amharic language from the research and publication office of the Jimma University)