EPIDEMIOLOGICAL STUDIES ON ASCARID INFECTIONS IN A CAT BREEDING UNIT

BY

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ABSTRACT

An epidemiological survey on the prevalence of ascariasis in a colony of breeding cats at the University of Edinburgh, Centre for Laboratory Animals, was carried out during the period between December 1978 and August 1979.

Routine helminthological techniques were used and egg counts performed. The animals under study comprised a population of 265 domestic cats including 178 adult males and females and 87 kittens.

Results showed that 22 out of 146 faecal samples contained Toxascaris leonina eggs. Isospora felis oocysts were also found in 20 out of the 146 samples, particularly among kittens.

Washings from the floors of the pens, cage washing machine filters and bed trays revealed contamination with ascarid eggs. Experiments on the development of T. leonina eggs showed that when in the artificial medium they developed quicker than in the faeces. Isoenzyme electrophoresis analysis of T. leonina adults using the enzyme glucose phosphatase isomerase showed differences between specimens from dogs and cats, and similarly extracts of T. leonina larvae from cats showed differences from adult worms from the same host species.

Treatment of individual animals with piperazine salts gave better results than treatment in groups.

Despite the prevalence of T. leonina eggs and coccidial oocysts among the samples studied, no significant clinical signs of infection were observed in the animals.

The results are discussed in relation to the epidemiology of the infection and suggestions are made for future control.

INTRODUCTION

Ascariasis in dogs (*Canis familiaris*) and cats (*Felis catus*) are commonly encountered. Heavy burdens can cause death of puppies and kittens and adult animals are unthrifty, emaciated or anaemic.

Roundworms, as the ascarids are normally known, infect a wide range of hosts, have a worldwide distribution and are important soil-transmitted parasites (W.H.O., 1964). Consequently any attempts to eliminate them and their sources of infection are fully justified.

The high prevalence of ascarid worms among dogs and cats is a salient feature in many helminthological surveys and these large parasites are usually present in significant numbers.

Helminthiasis in animals kept in captivity is a serious threat to the animal's health. Heavy infections are not uncommon in kennels and catteries, particularly when large numbers of animals are crowded together and sanitation is poor.

A large colony of breeding cats is maintained at the University of Edinburgh's Centre for Laboratory Animals at the Bush Estate. Garden (1978) examined 28 faecal samples from this colony and found *Toxascaris leonina* eggs in seven of these.

The purpose of the present work is to study the epidemiology of the ascarid infections in this colony of breeding cats in greater detail and to make suggestions for future control measures.

LITERATURE REVIEW

A. ASCARIDS OF CANIDAE AND FELIDAE

1. INTRODUCTION. From a study of the literature, one can easily conclude that helminth diseases are important in domestic animals.

Small animals, such as dogs and cats are not exceptions and are commonly found infected by harmful parasites. Among them, ascarid worms can cause significant economic losses, public health problems and are important soil-transmitted helminths (W.H.O., 1964).

The taxonomic position of these ascarids of dogs and cats is described by Soulsby (1978). They are nematodes belonging to the Order Ascaridida. Toxascaris leonina is included in the Family Ascaridae, and Toxocara canis and Toxocara cati in the Family Anisakidae.

As they resemble each other so closely in external appearance, confusion has occurred in the past regarding their host specificities and distribution (Sprent, 1959).

Heavy infections in dogs and cats are commonly encountered in places where large numbers of animals are kept together, such as kennels and catteries (Jacobs & Prole, 1976; Merz-Schenker, Freudiger & Horning, 1976).

In light infections, animals show general unthriftiness associated with intermittent diarrhoea. Acute infections cause digestive disturbances and sometimes nervous signs and death, particularly in puppies and kittens (Soulsby, 1965).

The importance of ascarid worms of dogs and cats in human health has been studied by several authors and Beaver (1956) concluded that

T. canis is the most important ascarid involved in human visceral larva migrans.

2. MORPHOLOGY. The main characteristic features used to differentiate the three species are based upon the presence and shape of cervical alae, position of genital organs, tail in the male, presence or absence of oesophageal ventriculus and eggs (Dunn, 1969; Giorgi, 1969).

Soulsby (1965) includes another difference in that Toxocara species tend to flex the anterior end ventrally and Toxascaris species dorsally. Okoshi and Usui (1967a), however observed many specimens not showing this feature. They also found it almost impossible to differentiate them by size alone. Dunn (1969) stressed that the cervical alae are important morphological features which enable one to distinguish the three species. In T. cati they are broad and short, whereas in T. canis and T. leonina they are narrow and long. However, no differences between the cervical alae of T. canis and T. leonina were found by Okoshi and Usui (1967a) but these authors concluded that the distance between transverse striations in the cuticle of T. leonina is distinctly shorter than in T. canis and T. cati.

The presence of an oesophagus ventriculus is pointed out by Giorgi (1969) as a salient feature to differentiate immature adults of Toxocara from Toxascaris.

The eggs of T. leonina are clearly distinct from those of Toxocara species. They have a smooth egg-shell and a transparent appearance. Toxocara eggs are thick with dark pitted shells (Soulsby, 1978).

Giorgi (1969) pointed out that T. leonina eggs and Isospora felis oocysts are very similar in shape but that the oocysts are less than

half the size of the Toxascaris eggs.

Several authors have shown that there are different strains of T. leonina occurring in canines and felines. Sprent (1959) comparing adult worms of T. leonina derived from dogs and wild canines found that the oesophagus/length ratio in the lynx (Lynx canadensis) was slightly greater than the others. Okoshi and Usui (1967b) in a similar investigation found that the body length and width in the canine specimens were greater than strains obtained from domestic cats, tigers (Panthera tigris) and cheetahs (Acinonyx jubatus).

3. HOSTS AND DISTRIBUTION. There is a general acceptance that three species of ascarid worms are found infecting Canidae and Felidae.

Canidae are the usual hosts of Toxocara canis Werner 1782, and Felidae of Toxocara cati Schrank 1788, whereas Toxascaris leonina von Linstow 1902 infects both Canidae and Felidae (Soulsby, 1965).

Among the principal hosts of T. canis are dogs, foxes (Vulpes vulpes) and wolves (Canis lupus) and very rarely Felidae. Sprent (1958) however referred to the occurrance of T. canis in two wild Felidae. Okoshi and Usui (1968a) succeeded in infecting dogs with T. leonina from canines, felines and wild Felidae, but Sprent (1959) failed to establish infection in cats with T. leonina eggs from dogs. Man is frequently infected with the larval stages of this species and the clinical manifestation known as visceral larva migrans has a world-wide distribution (W.H.O., 1964).

T. cati is more frequently encountered among Felidae and Sprent (1956) found no evidence of this species in Canidae. Somatic migration occurred in lambs (Sprent, 1956) and in chickens (Sprent, 1956;

Okoshi and Usui, 1968c). Sprent (1956) also found that cockroaches and earthworms could be infected by larvae of T. cati.

The host range of T. leonina is much wider than T. cati and T. canis. In the host list of T. leonina referred to by Sprent (1959), this species is found infecting the arctic fox (Alopex lagopus), the European wild cat (Felis silvestris) and a wide range of wild Felidae in Africa. Yamaguti (1961) has listed several species of Toxascaris infecting different hosts, particularly Ursidae. Several names have been proposed for these, such as Toxascaris transfuga, T. melursus, T. multipapillata and Sprent (1968) considered all belong to one species which is now known as Baylisascaris transfuga.

Ascarid eggs are extremely resistant to environmental conditions. Okoshi and Usui (1968b) showed that T. leonina eggs when stored at -15°C for 40 days developed normally. Antonenko (1976) found that 8-30% of Ascaris eggs were viable after 13 months in litter-free liquid manure. This could explain the wide distribution of these parasites.

Several studies in temperate areas indicated an infection rate with T. canis of about 20% and it is possible that in some tropical regions this percentage is even greater (W.H.O., 1967). In Britain, Woodruff (1976) concluded that 12% of dogs and cats are infected with ascarid worms, as well as up to 5% of apparently healthy people. In another survey carried out in Glasgow by Girdwood, Quinn, Bruce and Smith (1976) 32% of the female dogs examined harboured adult Toxascaris and Toxocara. Visco, Corwin and Selby (1978) examined 1,294 faecal samples from cats in the U.S.A. and found 24% infected with ascarid eggs and 6.7% infected with coccidia oocysts.

Infections with ascarid worms are a common finding in Felidae and Canidae kept in captivity. Surveys performed at Zoological Gardens usually reveal the presence of both Toxocara and Toxascaris species. In a recent epidemiological study at the Edinburgh Zoological Park, Garden (1978) found 14 Felidae infected with T. leonina and 2 Scottish wild cats (Felis silvestris grampia) with T. cati.

As previously stated, colonies of breeding animals, such as kennels and catteries are excellent sites for the rapid spread of ascaris infections. Jacobs and Pegg (1976) examining 574 faecal samples of elite show dogs, found 7.5% infected with T. canis and 2.8% with T. leonina. From faecal samples collected in a cat laboratory colony in the U.S.A., Guterbock and Levine (1977) found T. cati eggs in 32%, T. leonina in 6%, I. felis in 23% and Toxoplasma gondii in 1%.

4. THE LIFE CYCLE. Although Toxocara and Toxascaris belong to the same Order Ascaridida, their life histories and relationships with their hosts show important differences.

They are found in the small intestine and it has been estimated that one single mature female of T. canis may produce about 200,000 eggs per day (Douglas and Baker, 1965). Under satisfactory conditions of temperature (15°C to 35°C) and oxygen supply the eggs develop to the infective stage within a period of 3-6 days for Toxascaris and 2-4 weeks for Toxocara. At this stage they contain the second-stage larva (Soulsby, 1965).

The phenomenon of hatching ascarid eggs in vitro has been studied in detail by several authors (Rogers, 1960; Fairbairn, 1961; Pitts, 1963). They concluded that the most important factor in stimulating

the hatching is the presence of dissolved gaseous carbon dioxide and undissociated carbonic acid. Reducing agents, the addition of salts, variation in pH and temperature enhance or decrease the stimulus. Although these results stressed the importance of the carbon dioxide concentration, Cleeland and Lawrence (1962) achieved 90% hatching of A. suum eggs simply by gentle agitation of egg suspensions with a teflon bar magnet.

After hatching in the stomach, larvae of T. canis and T. cati penetrate the intestinal wall and migrate by the portal bloodstream to the liver and by the posterior vena cava to the lungs. They then migrate up to the pharynx and are swallowed. This type of life cycle is known as tracheal migration (Dunn, 1969). However with T. leonina in both dogs and cats the migration in the tissues is of a local somatic nature being into the wall of the intestine (Soulsby, 1965). Sprent (1959) showed that T. leonina adults are found in the intestinal lumen after 28 days and eggs appear 74 days after infection.

However, if the infective eggs of T. leonina are ingested by an abnormal host they remain as second stage larvae in the intestinal wall for about one week and then migrate through several somatic tissues (Sprent, 1952). Such larvae when they regain their normal host are capable of developing in the wall or the lumen of the intestine to reach maturity. Successful infections were achieved by Sprent (1959) by feeding final hosts with infected tissues from mice. He concluded that as T. leonina completes the life cycle within the intestine wall intermediate hosts are not fully necessary.

Okoshi and Usui (1968c) failed to infect cockroaches and earthworms with eggs of T. leonina, but succeeded in infecting earthworms

with T. cati and T. canis eggs.

The life-history of T. cati was studied by Sprent (1956) and Okoshi and Usui (1968c). These authors observed that somatic migration occurred in mice, lambs, chickens, earthworms and cockroaches. They concluded that this species is well adapted to use intermediate hosts.

It has been shown that in dogs which were infected with T. canis when over 5 weeks old, no adult worms could be found in the alimentary tract, but were scattered throughout somatic tissues (Sprent, 1958). It was concluded that this deposition of larvae in the tissues predisposes to prenatal infection; whereas the behaviour of T. cati predisposes to direct infection in cats.

It has been found that in bitches infected at different times of pregnancy, the invasion of foetuses by T. canis larvae occurs after the forty second day of gestation (Douglas and Baker, 1965). Sprent (1958) postulates that somatic migration of T. canis in dogs is an adaptation to the non-predatory habits of this animal.

Okoshi and Usui (1968c) pointed out that humans could act as paratenic hosts for the three ascarids of dogs and cats.

In a discussion on the evolution of the Ascaridoidea Sprent (1962) speculates that the primitive life-history of these nematodes could be compared to a pyramid. The base of such a pyramid would be occupied by a wide range of invertebrate hosts harbouring second-stage larvae and the apex by the dominant predator with its sexually mature parasite.

5. DIAGNOSIS. The presence of eggs, larvae or adult worms in the faeces or in the environment surrounding animals is an indication

of the presence of helminths. Faecal egg counts must be interpreted with caution and many different factors can limit the accuracy of results (Ministry of Agriculture, Fisheries and Food, 1977).

The techniques used to diagnose ascariasis are based on the recognition of the characteristic eggs in the faeces. Direct smears can be useful in quickly assessing prevalence data but have the disadvantage that high concentration of eggs is needed and quantitative results are not obtained. This method has been used extensively in man (W.H.O., 1967). To eliminate some of these problems, several methods involving salt flotation are in current use (Sewell and Hammond, 1978, unpublished).

Comparing several different methods, Okoshi and Usui (1967c) found that the saline flotation method and the anti-formin ether method gave lower results, although the eggs were more easily seen in the former method. They considered that the direct smear method was satisfactory for diagnosis in clinical cases, but recommended that three examinations of one faecal specimen should be made.

Soil plays an important role in the epidemiology of ascariasis infections (W.H.O., 1967). Consequently measurements of soil and environmental contamination are very important, particularly in places where large numbers of animals are crowded together. A technique for the quantitative recovery of ascarid and other nematode eggs from soil was demonstrated by Sewell and Urquhart (1976). Garden (1978) described a modification of this technique for application in places where it was not possible to obtain soil or vegetation samples. Washings from rock dens and concrete surfaces at the Edinburgh Zoo were obtained by pouring fluid over the selected area; it was then stirred by hand and

collected by using a brass syringe and stirrup pump. The flotation fluid which contained 1% Tween 80 was then centrifuged and the supernatant washed first through a sieve of 150 μ pore size and into one of 56 μ pore size which retained the eggs. This technique gave good results for egg recovery.

The differentiation of larvae from nematodes of animals and man is given in a key by Levine (1968).

Adult worms of T. leonina and T. cati can be differentiated by the shape of the cervical alae but these are almost indistinguishable in T. leonina and T. canis (Dunn, 1969). Studying toxascarids obtained from dogs, cats, tigers and cheetahs, Okoshi and Usui (1967b) found that differences were observed both in the morphology of the worms and the size of the eggs from the species examined. Despite these differences, they concluded that they should be unified into one single species, T. leonina.

Marked distension of the abdomen and poor physical condition are suggestive of ascarid infections in puppies and kittens. These signs should be checked by finding immature or mature worms in the vomit or faeces, or eggs in the faeces before a positive diagnosis can be made (Levine, 1968).

Several serological studies have been made on the diagnosis of ascariasis, particularly Toxocara infections. The immunofluorescent antibody technique has been used recently in the diagnosis of human toxocaral larva migrans by Viens, Strykowski, Richards and Sonea (1975), and in Toxocara infection in pigs by Stevenson and Jacobs (1977). Glickman and Cypess (1977) using the ELISA test detected antibodies to T. canis in 7 of the 60 people exposed to dogs and in one of the 13 unexposed ones.

Enzyme electrophoresis has been shown to be a useful technique in the species identification of helminths. Different electrophoretic patterns of the enzyme malate dehydrogenase were demonstrated by Zee and Zinkham (1975). These authors found that T. canis and T. cati exhibited identical patterns but these differed from those of T. leonina. Le Riche and Sewell (1977) using the enzyme glucose phophatase isomerase (G.P.I.) succeeded in differentiating Taenia saginata from Taenia solium. Garden (1978) using G.P.I. showed that a difference also exists between T. leonina and T. cati and that Baylisascaris transfuga also differed from both T. leonina and T. cati.

The effects of T. leonina in dogs and cats are similar to those of T. canis except that as there is no migration to the lungs pneumonia and prenatal infections do not occur (Dunn, 1969).

Eosinophilia is common in dogs infected with ascarids and El Hindawy (1948) found that the percentage of eosinophils in the peripheral blood may reach 36.5%. This author stated that high eosinophil counts are associated with chronic infection and not neccessarily with heavy ones. Ronéus (1971) studying the inter-relationship between the number of orally administered A. suum eggs, blood eosinophilia and the number of adult intestinal ascarids, concluded that the development of adult worms seemed to be impeded by high levels of blood eosinophils.

6. IMMUNITY. Little information could be found in the literature but it seems that puppies and kittens are much more susceptible to infection than older animals and patent infections are much less common in adults. Vasilef (1961) showed that dogs which were infected by either embryonated eggs or larvae encysted in rabbit tissue, acquired immunity only developed in those animals infected with eggs. Dodson,

Campbell and Webb (1967) found anaphylactic and reagin-like antibodies in rats infected with T. canis larvae. Hogarth-Scott (1967) showed that the molecular weight of the allergenic components of T. canis, T. cati and T. leonina ranged from 10,000 to 50,000 and suggested that these nematodes can provoke a Type I allergic reaction. Some helminth infections, known to induce hypersensitivity mechanisms also stimulate an IgE increase. This has been shown for Toxocara in humans by Hogarth-Scott, Johansson and Bennich (1969).

Vitamin deficiencies in some cases lower the resistance to infections with intestinal nematodes quite markedly (von Brandt, 1973). A lowered resistance of vitamin A-deficient hosts has been described for infections with T. canis and T. leonina by Wright (1935).

Beaver (1969) suggested that encapsulation of the larvae is a reaction favourable to the parasite and not a common type of foreign body response, providing comfort and needs for survival of the parasite and allowing it to live unmolested, with its infectivity fully preserved for months or years.

7. PREVENTION, CONTROL AND TREATMENT. Sanitation is the most important measure in either controlling or preventing ascariasis. Although it is not difficult to cure an individual patient with ascarid infections by drug medication, success in controlling the sources of infection is not promptly achieved (Giorgi, 1969). As infection depends mainly on the ingestion of infective eggs, control measures should be directed toward the prevention of the contamination of food and water by faecal material.

Ascarid eggs are typically very resistant to environmental

conditions and as stated by Giorgi (1969) the standard laboratory procedure for obtaining infective larvated eggs involves incubation in diluted formalin.

Some compounds are reported in the literature to destroy ascarid eggs and Stede (1971) has described the effect of "Lysococ" against T. leonina eggs. El Moukdad (1977) found that carbon disulphide compounds such as "Dekaseptol 6%" and phenol derivatives such as "Incidin-Anticoc 6%" killed T. leonina eggs by treatment in 30 minutes and one hour respectively. Pegg (1977) has recently reported that the use of a horticultural flame gun reduced T. canis infections on concrete-floored kennel runs, but Garden (1978) using the same procedure in an infected puma pen at the Edinburgh Zoo had disappointing results.

Several anthelmintics can be used in the treatment of ascarids of dogs and cats and Alexander (1976) described the pharmacology of a number of such drugs.

Santonin and oil of chenopodium were used for many years in the treatment of ascarid infections and the latter can be particularly poisonous to cats (Alexander, 1976). This author considered that piperazine is one of the most important anthelmintics developed in recent decades. It appears to act by depressing motility so that the worm cannot maintain its position in the intestine and is expelled by the peristaltic movements of the gut. Mann, Harfenist and Beer (1955) gave a series of daily doses of piperazine citrate to eleven cats at a rate of 100mg/Kg. They found that all the worms had been passed by the 4th day. Greenberg, Seymour and McEwen (1958) reported vomiting following high doses of piperazine in puppies but that kittens were less sensitive than puppies. Kirk (1977) considered that piperazine

compounds are 80% efficient.

Comparing several drugs, Okoshi and Usui (1967c) found that piperazine adipate was 100% effective against T. leonina in cats but that immature worms were more resistant than adults.

Many other anthelmintics such as Dichlorvos, Diethylcarbamazine, Thenium and Mebendazole (Giorgi, 1969) have been used with good results against ascarids. Dichlorvos and pyrantel pamoate were considered by Kirk (1977) to be 95% efficient in the treatment of ascarids of dogs and cats, and Scupin, Brandes, Niessen and Scupin (1976) described the efficacy of pyrantel pamoate paste given orally. Stilbazium iodide has been used in the treatment of ascarids of dogs and cats by Burrows and Lillis (1962) and T. leonina and T. cati were almost completely eliminated with 2 doses of 25mg.

Because the infective eggs of ascarids are very resistant to chemical agents, good sanitation and management of animals appear to be the best way of controlling infections by ascarids in kennels and catteries (Levine, 1968). Therefore, if the faeces are not allowed to accumulate for more than about a week, the probability of the animal ingesting infective eggs would become quite small particularly with T. leonina and T. cati where infection leads directly to patent infection (Giorgi, 1969).

B. COCCIDIOSIS OF DOGS AND CATS

Intestinal protozoa are frequently detected in dog and cat faeces. However coccidia are thought to be pathogenic only if another disease is also present or in weakened hosts (Giorgi, 1969).

Occasional outbreaks of coccidiosis may occur from time to time in catteries where sanitation is poor and where animals are crowded together (Wilkinson, 1977). Stewart (1947) stated that the disease is frequently initiated by a carrier animal kept in insanitary conditions with a number of other animals, but unhygienic conditions are however not necessary and the use of disinfectants may favour the survival and development of coccidial oocysts by reducing the bacterial flora.

It is generally accepted that the classification of coccidia is based mainly on morphological features and biological characteristics. Thus Levine (1961) grouped them mainly according to the number of sporocysts or sporozoites formed during sporulation, while Wenyon (1965) classified the coccidia on the biological phenomena observed during the stages of development.

Infection in dogs and cats is generally due to coccidia of the genus Isospora. In this genus two sporocysts each with four sporozoites may be seen in the sporulated oocysts (Levine, 1961).

Three species of Isospora have been reported as occurring in the cat. Isospora felis, which is probably the most prevalent particularly in young cats, Isospora bigemina, which is rare but more pathogenic, and Isospora rivolta, which is not very prevalent (Wilkinson, 1977).

Measurements of oocysts and sporocysts of the genus Isospora are given by several authors (Wenyon, 1965; Levine, 1961; Pellérdy,

1965), and some discrepancies are apparent. Pellérdy (1965) stated that even the most elaborate classification is provisional, since the continuous increse of knowledge necessitates repeated revisions.

Wenyon (1965) described two types of oocysts of I. bigemina. The smaller ones measure 10 to 14 µ by 7.5 to 9 µ and occur in the dog and the cat and the larger ones measure 18 to 20 µ by 14 to 16 µ and are found only in the dog. This same author stated that the large and small oocysts probably belong to different species. Recent workers (Hutchison, Dunachie, Work and Siim, 1971; Ito, Tsunoda, Nishikawa and Matsui, 1974) have shown that the small oocysts are produced by the coccidian parasite Toxoplasma gondii.

Hutchison et al (1971) compared and contrasted the coccidian stages of T. gondii with those of Isospora species in the cat. Despite the significant resemblances between the two parasites, they supported the retention of the existing nomenclature of T. gondii. Dubey and Frenkel (1972) presented evidence that I. felis and I. rivolta invade the extra-intestinal tissues of cats and that these stages may be confused with Toxoplasma.

The measurements of oocysts and sporocysts of T. gondii quoted by Hutchison et al (1971) from the work of several authors have a range of 10-20 µ by 5.5-16 µ and 7-15.5 µ by 3-10 µ respectively.

Hitchcock (1955); Dubey and Strietel (1976) infected young cats with 100,000 sporulated oocysts but did not observe marked clinical signs. According to Pellérdy (1965) I. felis settles in the superficial epithelium. Its endogenous stages are located usually above the nucleus in the epithelial cells. Only large infective doses are fatal. In such cases, haemorrhages, ulceration of the mucosa and diarrhoea are seen.

Reciprocal effects of Toxoplasma and Isospora have been shown by several authors. Campana-Rouget, Dorchies and Gourdon (1974) reported that administration of T. gondii cysts or oocysts to kittens naturally infected with I. felis caused reactivation of the I. felis infection and almost total inhibition of the T. gondii. However in a similar experiment Chessum (1972) found that a latent T. gondii infection in a cat was reactivated by the administration of 100,000 oocysts of I. felis.

The diagnosis of coccidiosis is based on the clinical signs and the presence of large numbers of oocysts in the faeces but as stated by Stewart (1947) the diagnosis is not always easy and cannot in all cases be related to the number of coccidia seen in a single observation. Clinical findings and the history of the kennel or cattery have to be considered together with laboratory reports.

Few reports have been found in the literature on the treatment and control of Isospora infection. However Wilkinson (1977) reported that sulphadimidine appeared to have no effect on the number of oocysts in the faeces but sulphadimethoxine produced a reasonably rapid elimination of oocysts from faeces with only minor side effects.

Coccidial oocysts are considered to be markedly resistant to disinfectants. El Moukdad (1976) however succeeded in inhibiting sporulation of some species of coccidia by using carbon disulphide compounds and phenol derivatives. A 10% solution of formaldehyde was ineffective against unsporulated oocysts.

Pellérdy (1965) considered that where animals are kept together in considerable numbers, control measures should include regular cleaning of the runs and frequent replacement of litter.

MATERIAL AND METHODS

1. LOCATION. The University of Edinburgh maintains at the Centre for Laboratory Animals, Bush Estate, Roslin a colony of breeding cats. The present study was conducted there from December 1978 to August 1979. All samples were processed and experiments carried out at the Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian.

2. THE CAT COLONY. The building is composed of two floors harbouring approximately 260 domestic cats. On the ground floor there are two nursery rooms, of 14 x 2 metres identified as the Red Room and the Blue Room (Fig.1), where the queens are kept in individual cages with their litters. On the same floor is located the washing up room where a cage washing machine cleans and rinses the cages and sanitary trays (Plate 8).

On the first floor there are six rooms divided in two pens each of 6 x 2 metres for growing and breeding animals (Fig. 2). For the purposes of the present study the pens in each room were identified as A and B and this floor also contains a special room for food preparation.

3. MANAGEMENT. The average number of animals per pen is between fifteen and twenty, with one adult male for each fifteen females in the breeding pens. However the total number of cats per pen fluctuates at different periods of the year according to the availability of animals.

To prevent the introduction of disease the colony is a closed unit and no animals from outside are introduced.

Two people are directly responsible for daily management of the colony and they feed the animals and clean the pens.

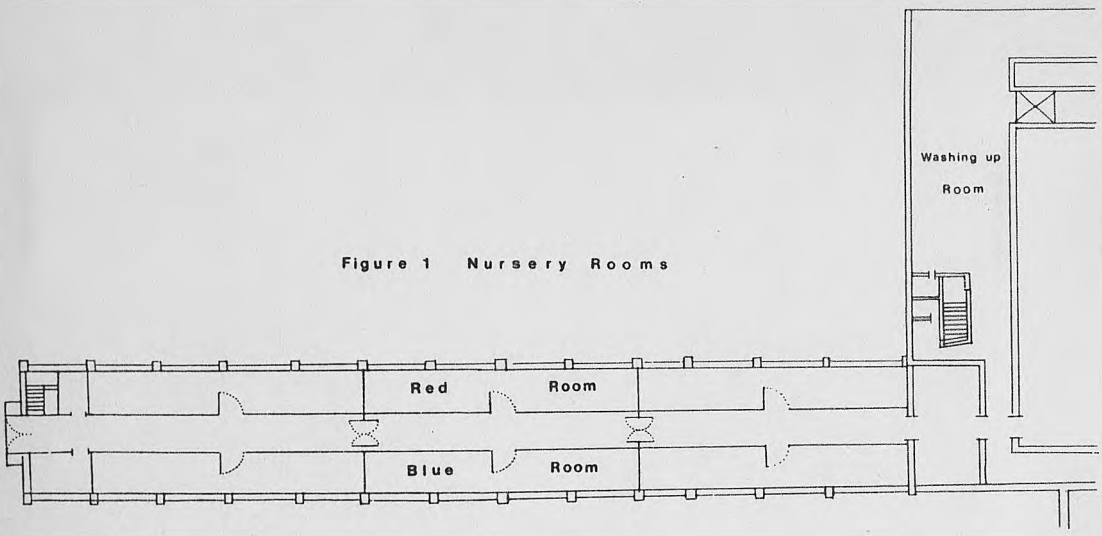
The pregnant queens give birth to their litters in individual cages in the nursery rooms (Plate 1). The kittens are weaned at 8 weeks of age. At that time the mothers return to the breeding rooms. At 8, 10 and 12 weeks of age the kittens are given 0.5 g piperazine citrate (Citrazine powder, J.M. Loveridge, Southampton) in the food. When 12 weeks old all kittens are transferred to the growing rooms (Plate 4). Their cages and sanitary trays are washed and rinsed in the cage washing machine (Associated Crates Ltd., Stockport, England). While in the machine they are washed by jets of water at 90°C for 2 minutes 45 seconds (Plate 8).

All kittens are protected against feline panleucopenia (infectious enteritis) with the Katavac P vaccine (Duphar Veterinary Limited, Southampton).

Each pen is provided with two sanitary trays made of aluminium which contain a sufficient amount of wood shavings (Plate 5). These trays are changed every two days. The asphalt floors of the pens are washed every three weeks with a 1% cleaner disinfectant (Novaklene, Dextar Chemicals Limited, Lockerbie, Dumfriesshire) after the faeces have been removed. All pens are provided with drainage sumps.

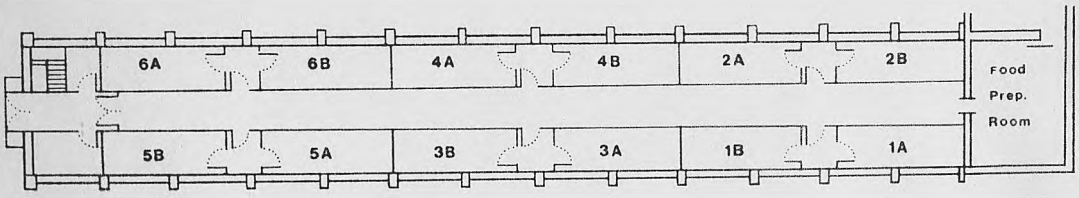
The food is composed of frozen cattle meat, milk powder, fish meal and maize flakes. The ingredients are mixed up and given twice a day in plastic containers to the animals in the nursery rooms and in metallic containers to those in the pens. Water is available at all times in plastic bowls. All food and water containers are washed daily in hot water with 5% of soft detergent (A. Beverage, Edinburgh).

Figure 1 Nursery Rooms



Ground Floor

Figure 2 Common Rooms (Pens)



First Floor

PLATE 1

Individual cage in the 'nursery room'.

Note kittens sleeping in the sanitary tray.

PLATE 2

Individual cage in the 'nursery room'.

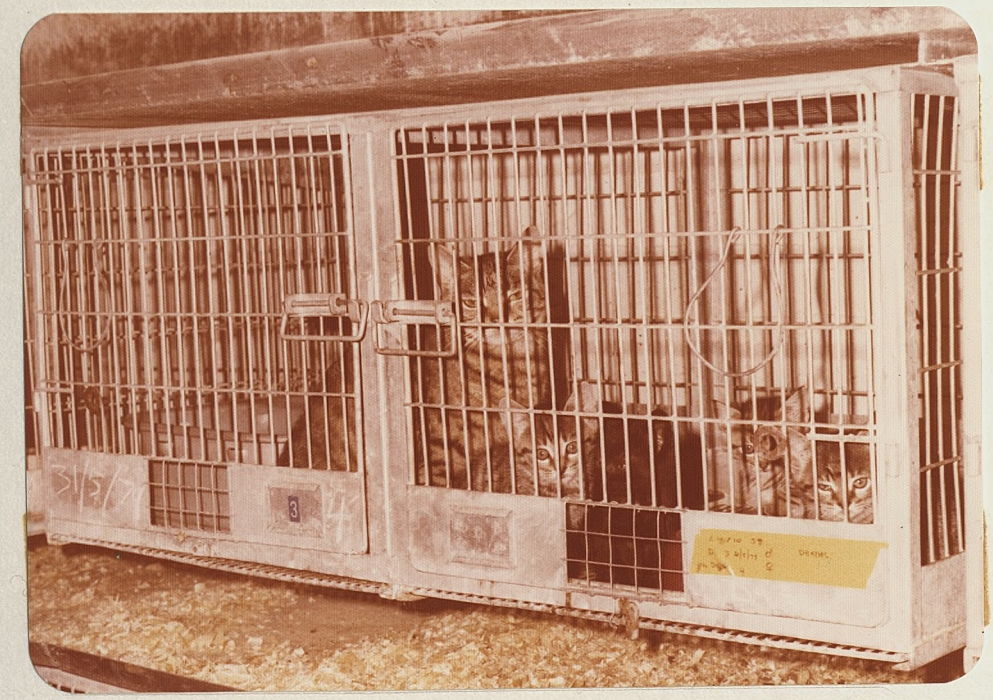


PLATE 3

Individual cages in the Blue room.

PLATE 4

Kittens over 12 weeks old in the 'growing room'.

PLATE 5

Adult cats in pens in a 'breeding room'.

Note cats resting in sanitary trays; faeces on the floor.

PLATE 6

Treatment of kittens.

Note four animals not eating.

PLATE 7

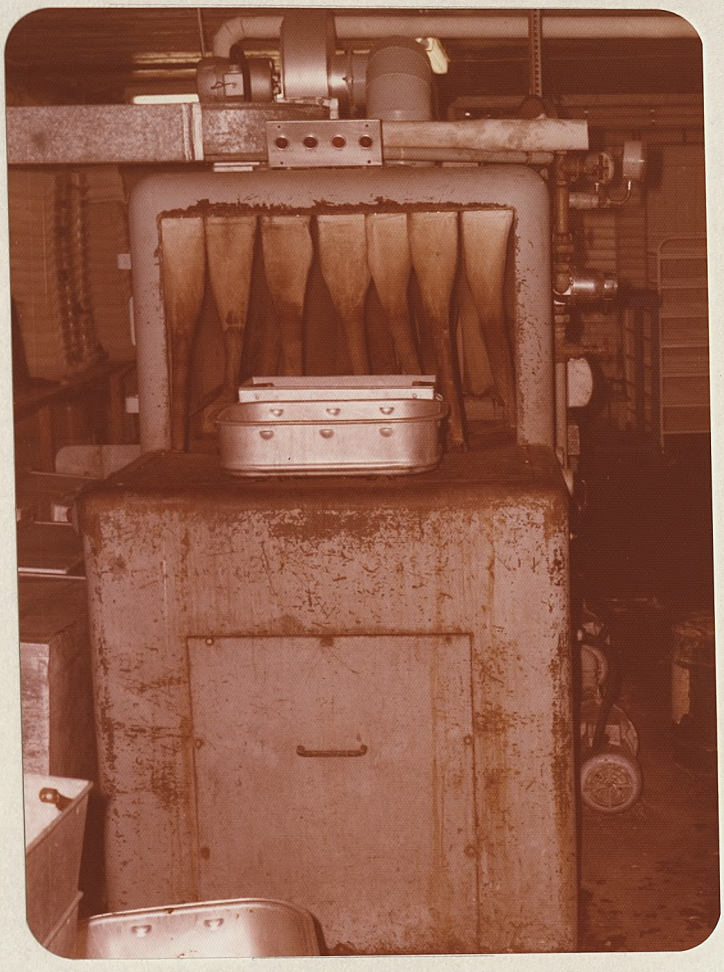
A breeding pen.

PLATE 8

Cage washing machine.

PLATE 9

Cage washing machine showing two filters.



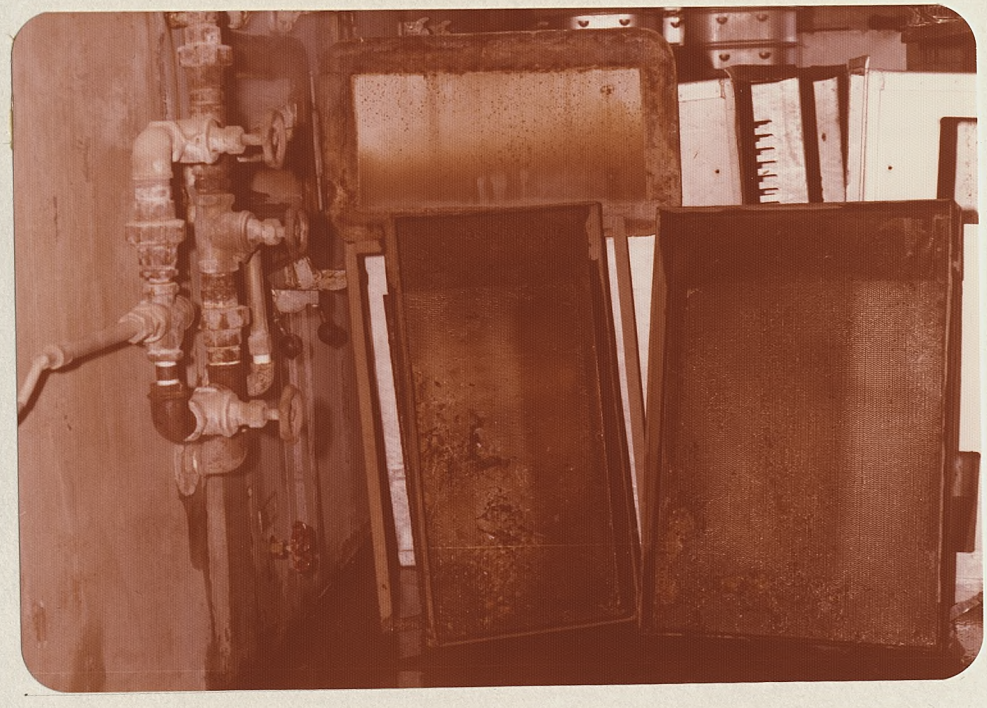


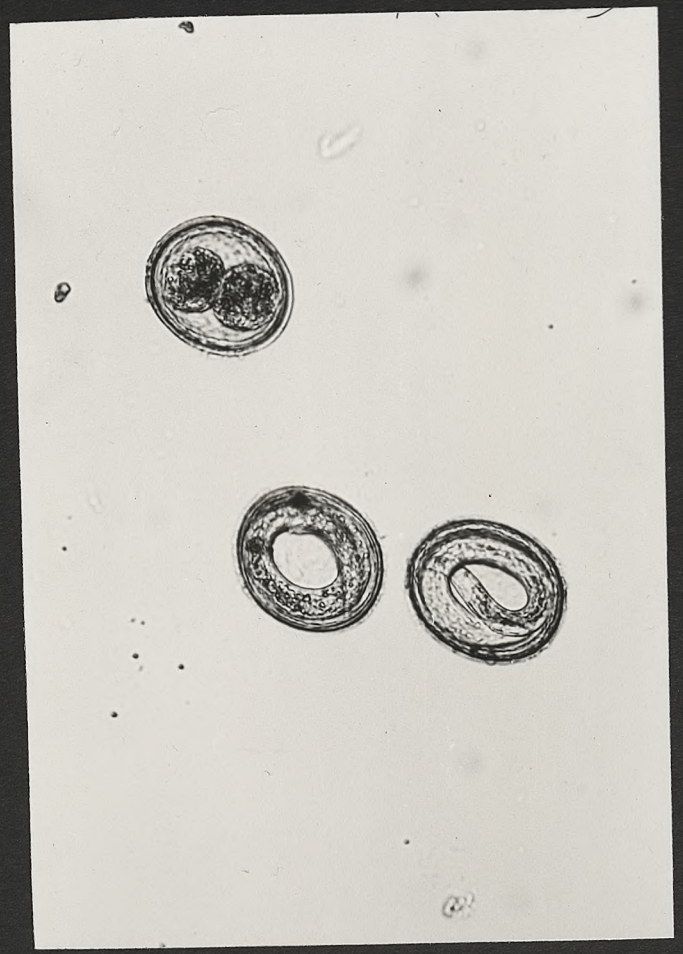
PLATE 10

Eggs of T. leonina, 280 x.

PLATE 11

Eggs of T. leonina, 280 x.

Two larvated eggs.



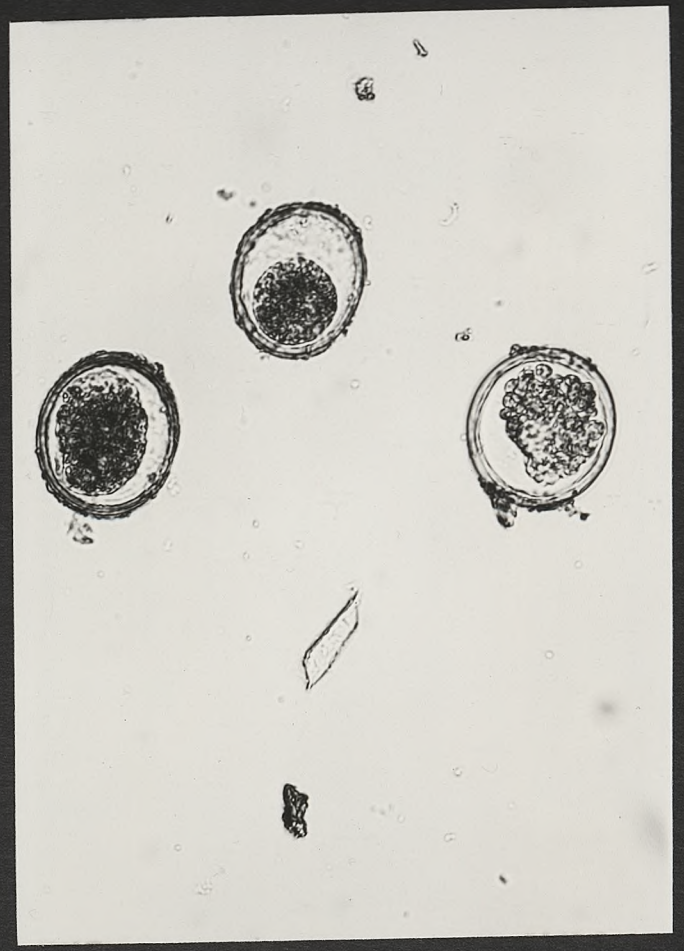


PLATE 12

Eggs of *T. leonina*, 280 x.

One egg hatching.

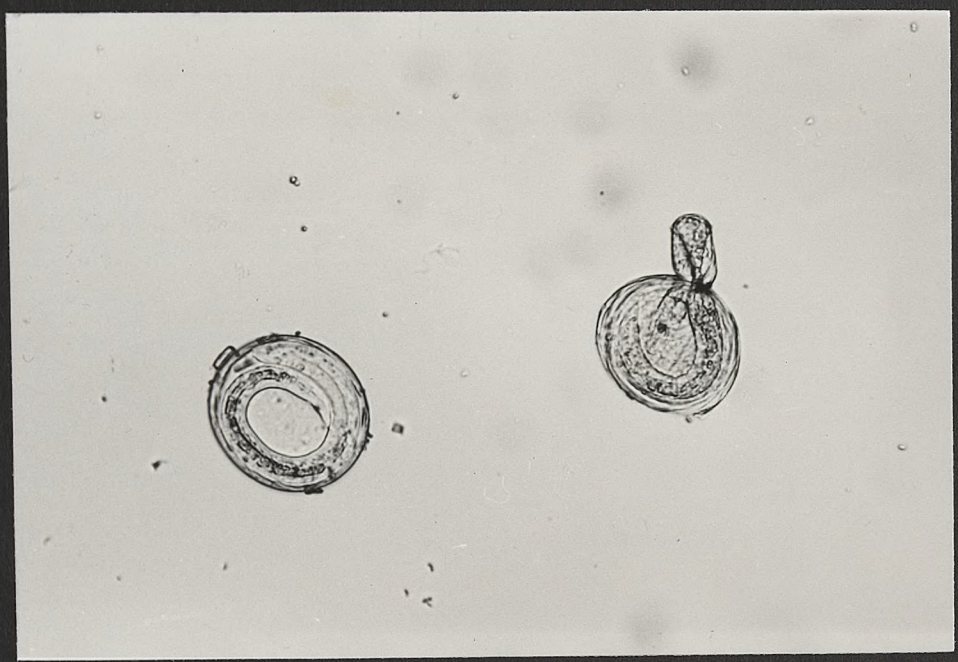
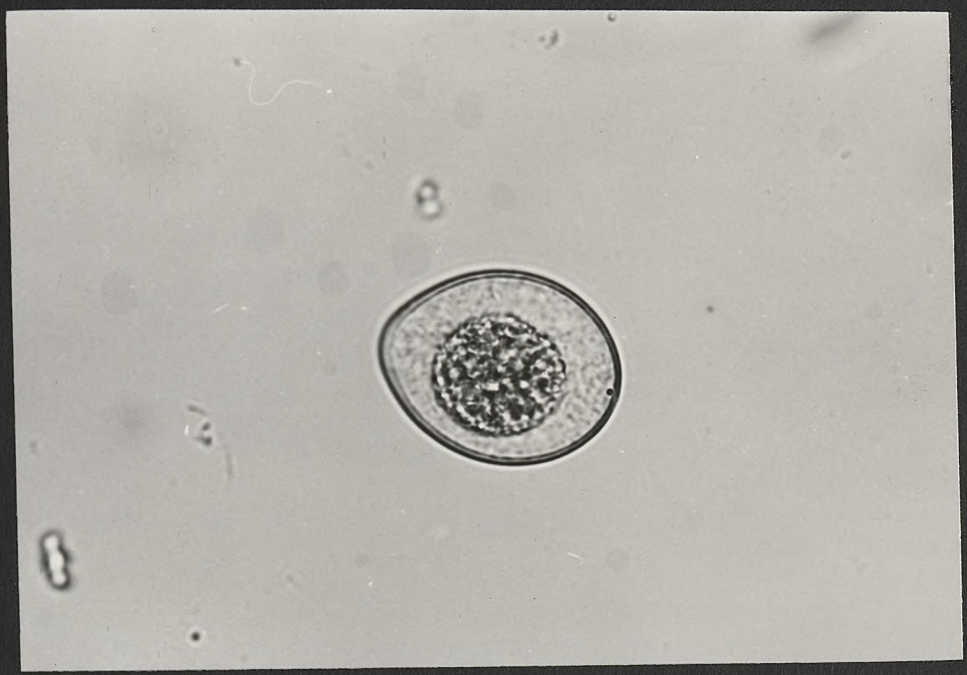


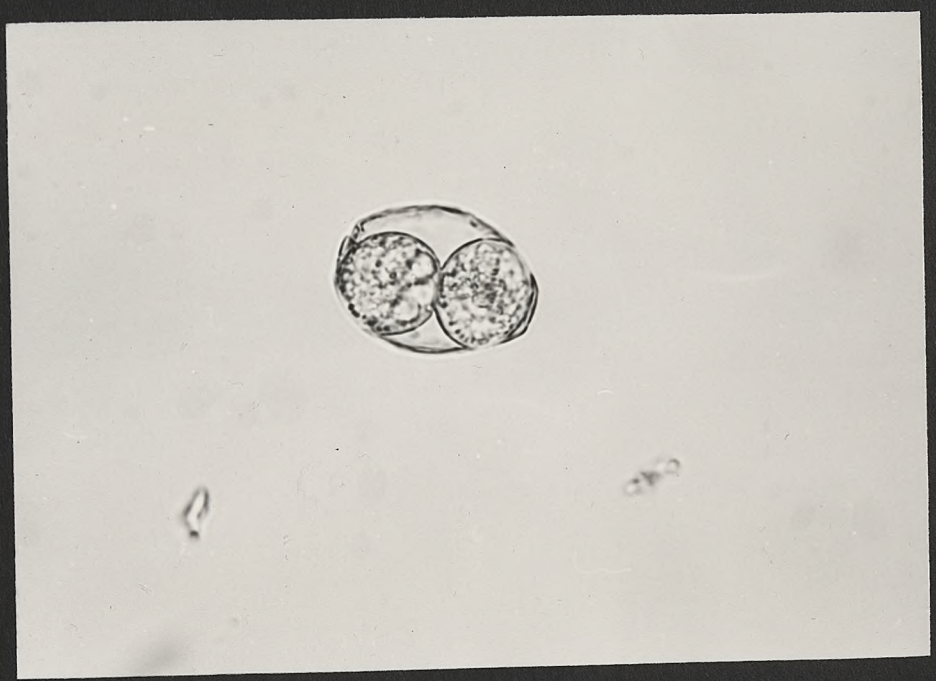
PLATE 13

Oocyst of *I. felis*, 720 x.

PLATE 14

Oocyst of *I. felis* (sporulated), 720 x.





Cats over 12 weeks old are treated against ascarids with 1g piperazine citrate per cat mixed in the food once a month (Plate 6).

At the beginning of the study the total number of animals was 265. There were 29 queens with 87 kittens on the ground floor and the first floor contained 149 adults including males and females.

4. SAMPLING AND EXAMINATIONS. (i) FAECES. Faecal specimens were collected in plastic containers, labelled and stored at 4°C. Although an attempt was made to collect samples once a week it was not always possible to obtain fresh samples from all the pens. At least two specimens were collected from each pen. Most of the individual cages were also sampled as time permitted. Nine faecal samples from wild carnivores were collected at the Edinburgh Zoo and examined for T. leonina eggs in order to compare them with those from domestic cats. Three cadavers of cats were obtained from the Cat and Dog Home, Seafield Road, Edinburgh and autopsied in an attempt to find T. cati.

All samples were initially screened by flotation in saturated salt solution using the technique described by Sewell and Hammond (Helminthological Techniques, 1978, unpublished). The Modified McMaster Method (Ministry of Agriculture, Fisheries and Food, 1977) was carried out on all those showing any parasite eggs or protozoa oocysts.

For the purpose of measurements, eggs and oocysts were recovered by the saturated salt flotation technique. A magnification of 500 x was used in a measuring chamber as described by Sewell and Hammond (1978, unpublished). Lengths and breadths of T. leonina eggs from domestic cats and a cheetah, and coccidial oocysts from domestic cats were measured and then compared by the method of minimum convex

polygons (Matthews, 1977).

(ii) FLOOR, SANITARY TRAY AND FILTER WASHINGS. Floor washings were collected by the author immediately after the routine washings performed by staff of the Centre. The technique used was a modification of the method for examining soil samples (Sewell and Hammond, 1978, unpublished). Areas which allowed fluid to accumulate were selected particularly the corners of the pens. A 1 litre mixture of saturated sodium chloride solution and 1% "Tween 80" (Polyoxyethylene Sorbitan Monooleate, SIGMA, London Chemical Company, POOLE) was poured over the selected area. The fluid was stirred by hand and the area was scrubbed with a small brush for 1 minute. The fluid was then collected using a 50 ml plastic syringe(Plastipak Becton, Dickinson Co., Republic of Ireland) to which a short plastic tube had been attached.

Four sanitary trays were each scrubbed with 100 ml of the same fluid just before being put into the individual cages.

The four filters from the cage washing machine were each scrubbed in 1 litre of the same fluid.

These washings were processed according to Sewell and Hammond (1978, unpublished) using a M.S.E. Mistral 4L centrifuge (Measuring and Scientific Equipment Ltd., Crawley). Eggs and oocysts were recorded as the number of eggs or oocysts per litre of fluid, except for the sanitary trays where they were recorded as eggs or oocysts per 100 ml of fluid.

5. TREATMENT OF CATS. Two infected cats were treated with piperazine citrate (Citrazine powder, J.M. Loveridge Ltd., Southampton). A dose of 0.5 g/kg body weight was administered in the feed in individual containers to assure that the cats ingested all the drug given.

One single treatment was applied. All faeces after treatment were examined for worms by the author. Specimens were placed in 0.85% saline for transport to the laboratory. Subsequent faecal examinations for the presence of eggs were performed.

6. THE DEVELOPMENT OF EGGS AND OOCYSTS. An experiment was set up in order to study the development of T. leonina eggs at different environmental conditions. Coccidial oocysts were sporulated in the laboratory.

(i) T. leonina eggs at laboratory conditions. The eggs were obtained from fresh faecal samples containing more than 2,000 e.p.g. They were separated and concentrated in several different batches by the saturated salt flotation technique. Batches showing eggs in the same stage of development were pooled. After three washings in 0.85% saline, the eggs were maintained in agar-formalin in Petri dishes as described by Okoshi and Usui (1968b) and observed for seven days. Results were recorded daily.

(ii) T. leonina eggs at the Centre for Laboratory Animals. An entire fresh faecal sample was collected from a known infected cat. This sample was sub-divided into seven aliquots, put in plastic containers, labelled 1 to 7 and kept in an appropriated tray. Each day for one week, one sample was collected and after separation as previously described, the eggs were examined and the results recorded.

In both experiments room temperatures were recorded daily and the rate of development of eggs was measured according to the method of Brown (1928). An index value was given to each stage of development (Table 1).

TABLE 1. INDEX VALUE AND STAGE OF DEVELOPMENT OF T. LEONINA EGGS FROM DOMESTIC CATS

INDEX VALUE	STAGE
0	1 cell
1	Early morula
2	Late morula
3	Tadpole stage
4	Motile embryo

One hundred eggs were examined daily on a McMaster slide and each stage of development recorded. Each percentage was then multiplied by the index value for the stage. The sum of these index values gives the total value for each stage and is a measure of the extent of development. Results were then expressed graphically.

(iii) I. felis oocysts. Faecal samples containing approximately 10,000 oocysts per gram were selected. The oocysts were separated, concentrated and sporulated in 2% potassium dichromate according to the technique described by Adam, Paul and Zaman (1971).

7. ENZYME ELECTROPHORESIS. Second stage infective larvae of T. leonina from domestic cats and adult Toxascaris and Toxocara were compared by enzyme electrophoresis.

Eggs of T. leonina obtained from infected faecal samples were separated, concentrated and allowed to become infective as previously described (Okoshi and Usui, 1968b; Sewell and Hammond, 1978, unpublished). The eggs were hatched by the technique described by Cleeland and Laurence (1962), but this procedure was abandoned as it was found to be

time consuming. Furthermore many larvae were found to be damaged by this technique and the released enzymes could have been destroyed by the incubation overnight at 37°C. Therefore the infective eggs were smashed up in a 3 ml tissue grinder (Mini Homogeniser, Jencons Scientific Ltd., Hemel Hempstead, Hertfordshire) (Sewell, 1979, personal communication). Larval extracts were prepared in a solution containing 1 mM EDTA (ED2SS, SIGMA Chemical Company, POOLE), dithiothreitol (0632 SIGMA) and ∝ amino-n-caproic acid (A2504 SIGMA).

Extracts from adult T. leonina from a dog, a cat and a puma (Felis concolor) and T. canis from a dog were obtained from the stock collection of the Helminthology Department of the CTVM.

The enzyme electrophoresis was carried out by the method described by Le Riche and Sewell (1977) using the following specifications:

(i) A thin layer starch gel with a thickness of less than 1 mm was prepared using 11% hydrolysed starch (B.D.H. Chemicals Ltd., POOLE) in 0.015 M phosphate buffer pH 7.4.

(ii) The electrode buffer was 0.2 M phosphate buffer at pH 7.4.

(iii) Electrophoresis was continuous at 330 volts for 3 hours and a 15 cm distance separated the terminals.

8. SEROLOGICAL TESTS FOR TOXOPLASMA ANTIBODIES. Four cats shedding Toxoplasma-like oocysts were selected by the author and bled by the staff of the Centre for Laboratory Animals. The indirect haemagglutination test was carried out, according to the technique described by Thorburn and William (1972), at the East of Scotland College of Agriculture, Veterinary Investigation Centre, Edinburgh.

9. FAECAL SAMPLES FROM KITTENS. An attempt was made to examine fresh samples from kittens between 8 and 12 weeks old. Ten kittens were sampled by rectal swabs. Each swab was smeared on a slide and transported to the laboratory. As the technique for sampling the animals was time consuming, this procedure was discontinued.

RESULTS

1. FAECAL EXAMINATIONS

Ascarid eggs were detected in 22 out of 146 samples (Table 2) and all were identified as T. leonina. Coccidial oocysts were found in 20 out of 146 samples (Table 3) and all were identified as I. felis. T. leonina was more prevalent in pens which housed the adult cats whereas I. felis oocysts were more prevalent in cages which housed both kittens and queens.

TABLE 2. DISTRIBUTION OF ASCARID EGGS IN FAECAL SAMPLES FROM PENS AND CAGES

	NUMBER SAMPLED	POS (%)	NEG	NUMBER OF SAMPLES	POS (%)	NEG
PENS	12	8 (66.6)	4	49	11 (22.4)	38
CAGES	42	11 (26.1)	31	97	11(11.3)	86
TOTAL	54	19 (35.1)	35	146	22 (15.0)	124

TABLE 3. DISTRIBUTION OF COCCIDIAL OOCYSTS IN FAECAL SAMPLES FROM PENS AND CAGES

	NUMBER SAMPLED	POS (%)	NEG	NUMBER OF SAMPLES	POS (%)	NEG
PENS	12	2 (16.6)	10	49	2 (4.7)	47
CAGES	42	18 (42.8)	24	97	18 (18.5)	79
TOTAL	54	20 (37.0)	34	146	20 (13.0)	126

The samples containing ascarid eggs obtained from sanitary trays in pens occupied by adult cats are shown in Table 4. The results of the salt flotation and McMaster techniques are both given.

TABLE 4. POSITIVE SAMPLES FOR ASCARID EGGS FROM SANITARY TRAYS IN PENS OCCUPIED BY ADULT CATS

DATE	ROOM/PEN	FLOTATION	McMASTER eggs per gram
5.12.78	1/B	MANY (1)	300
16. 1.79	2/A	MANY	3,100
16. 1.79	2/A	MANY	5,600
16. 1.79	2/B	FEW (2)	NIL
16. 1.79	2/B	MANY	100
16. 1.79	3/A	MANY	1,600
9. 2.79	3/A	MANY	11,900
16. 1.79	3/B	FEW	NIL
9. 2.79	4/B	MANY	100
16. 1.79	5/B	MANY	1,100
9. 2.79	6/A*	MANY	100

(1) More than 10 eggs per slide
(2) Less than 10 eggs per slide
* Pen occupied by younger cats (12-24 weeks old)

Although all cats in the pens were treated with piperazine citrate in the food on the first day of each month, it was possible to recover eggs from faeces 4, 8 and 15 days after treatment.

Large numbers of eggs were recovered from samples from ROOM/PEN 2/A, 3/A and 5/B. Larvated eggs were recovered only on one occasion, from 6/A.

The samples containing ascarid eggs obtained from sanitary trays in individual cages occupied by queens and kittens are shown in

Tables 5 and 6. The results of the salt flotation and McMaster techniques are both given.

TABLE 5. POSITIVE SAMPLES FOR ASCARID EGGS FROM SANITARY TRAYS IN INDIVIDUAL CAGES IN THE BLUE ROOM OCCUPIED BY QUEENS AND KITTENS.

DATE	CAGE NO.	FLOTATION	McMASTER eggs per gram
26.1.79	4*	MANY (1)	400
25.6.79	4	FEW (2)	300
7.2.79	7	MANY	200
9.2.79	8	FEW	NIL
25.6.79	13*	FEW	200
25.6.79	14	MANY	300
25.6.79	15	FEW	NIL

(1) More than 10 eggs per slide
(2) Less than 10 eggs per slide
* Only weaned kittens over 8 weeks old

TABLE 6. POSITIVE SAMPLES FOR ASCARID EGGS FROM SANITARY TRAYS IN INDIVIDUAL CAGES IN THE RED ROOM OCCUPIED BY QUEENS AND KITTENS.

DATE	CAGE NO.	FLOTATION	McMASTER eggs per gram
23.2.79	8	FEW (2)	NIL
23.2.79	11	FEW	NIL
30.1.79	15	MANY (1)	1,300
25.6.79	15	MANY	400

(1) More than 10 eggs per slide
(2) Less than 10 eggs per slide

The numbers of eggs recorded from individual cages were lower than from pens (Table 4).

All kittens were treated with piperazine citrate at 8, 10 and 12 weeks of age. Nevertheless in two cages in the Blue Room (No. 4 and No. 13) eggs were recovered from the faeces of 11 and 12 week old kittens. In the other cages, all kittens were less than 8 weeks old and consequently the queens were also present in the cages.

In spite of the number of positive samples in the Blue Room being greater than in the Red Room, the average number of eggs per cage recovered from the latter (425 eggs/cage) was more than twice the number recovered from the former (200 eggs/cage).

The samples containing coccidial oocysts obtained from sanitary trays in pens and individual cages occupied by adult cats, and kittens only are shown in Table 7, and the samples containing coccidial oocysts obtained from sanitary trays in individual cages occupied by both queens and kittens are shown in Table 8. The results of the salt flotation and McMaster techniques are both given.

TABLE 7. POSITIVE SAMPLES FOR COCCIDIAL OOCYSTS FROM SANITARY TRAYS IN PENS OCCUPIED BY ADULT CATS, AND INDIVIDUAL CAGES OCCUPIED BY KITTENS ONLY.

DATE	ROOM/CAGE or ROOM/PEN*	FLOTATION	McMASTER oocysts per gram
26.2.79	BLUE/ 3	MANY (1)	3,200
21.1.79	BLUE/ 4	MANY	100
25.6.79	BLUE/ 5	FEW (2)	100
7.2.79	BLUE/ 6	MANY	32,600
9.2.79	BLUE/ 8	FEW	NIL
25.6.79	BLUE/12	MANY	2,300
25.6.79	BLUE/13	FEW	2,000
26.2.79	BLUE/15	MANY	400
26.2.79	BLUE/16	MANY	1,200
9.2.79	BLUE/18	MANY	800
2.2.79	RED/ 1	MANY	1,400
23.2.79	RED/ 3	MANY	1,300
2.2.79	RED/ 6	MANY	1,800
26.2.79	RED/12	MANY	400
26.2.79	RED/18	MANY	600
14.5.79	1/A*	MANY	3,000
14.5.79	2/B*	MANY	2,500

(1) More than 10 oocysts per slide
(2) Less than 10 oocysts per slide
* Adult cats

TABLE 8. POSITIVE SAMPLES FOR COCCIDIAL OOCYSTS FROM SANITARY TRAYS IN INDIVIDUAL CAGES OCCUPIED BY BOTH QUEENS AND KITTENS.

DATE	ROOM/CAGE	FLOTATION	McMASTER oocysts per gram
26.2.79	BLUE/ 7	MANY (1)	1,200
26.2.79	BLUE/11	FEW (2)	300
25.6.79	BLUE/14	FEW	500

(1) More than 10 oocysts per slide
(2) Less than 10 oocysts per slide

Although much the highest count was from one cage (BLUE/6) occupied by kittens only, the mean number of oocysts recovered from the pens occupied by adult cats and from the cages occupied by kittens were similar. However the mean number recovered from the cages occupied by queens and kittens was much lower.

Both T. leonina and I. felis were recovered from one sample (2/B) from the pens (Tables 4 and 7), and from five samples (4,7,8,13 and 14) from the Blue Room (Tables 5, 7 and 8).

Where it was possible to identify the animals with ascarid eggs in their faeces, several samples were collected and the examinations were repeated. The results of these examinations in two cages from the Blue Room (No. 14 and No. 4) are illustrated in Graphs 1 and 2. Although all kittens were treated with citrazine immediately after being weaned, faecal samples collected from their sanitary trays remained positive.

T. leonina eggs were found in two of the nine faecal samples collected at the Edinburgh Zoo. The hosts and the number of eggs per gram are shown in Table 9.

TABLE 9. FAECAL EGG COUNTS (e.p.g.) IN WILD CARNIVORES.

HOST	T. LEONINA
LION (Panthera leo)	NEGATIVE
JAGUAR (Panthera onca)	NEGATIVE
TIGER (Panthera tigris)	NEGATIVE
HYAENA (Crocuta crocuta)	NEGATIVE
CHEETAH (Acinonyx jubatus)	2,100
PUMA (Felis concolor)	NEGATIVE
PUMA (Felis concolor)	300
LEOPARD (Panthera pardus)	NEGATIVE
RED FOX (Vulpes vulpes)	NEGATIVE

T. cati was found in two and Taenia taeniaeformis in one of the three domestic cats autopsied from the Cat and Dog Home.

Mean egg and oocyst parameters of T. leonina and I. felis are shown in Table 10.

TABLE 10. MEAN EGG AND OOCYST PARAMETERS OF T. LEONINA AND I. FELIS.

EGGS/OOCYSTS	LENGTH μ	BREADTH μ
T. leonina (domestic cats)	78.1 ± 2.53	67.8 ± 2.66
T. leonina (cheetah)	77.8 ± 5.27	67.9 ± 2.73
I. felis (domestic cats)	43.5 ± 2.74	33.5 ± 2.59

Measurements of T. leonina eggs from domestic cats and from a cheetah were recorded for comparison. Individual egg and oocyst parameters are listed in Appendix Tables I-III and are illustrated by the method of minimal convex polygons (Graph 3-6). Graph 6 shows that

the polygons of T. leonina from both domestic cats and the cheetah overlap.

Sporulation of the oocysts at room temperature was completed in about 72 hours and four sporozoites developed in each sporocyst.

2. FLOOR, SANITARY TRAY AND FILTER WASHINGS

Results from examination of washings from floor, sanitary trays and cage washing machine filters are shown in Table 11.

TABLE 11. RESULTS OF EXAMINATION OF WASHINGS FROM FLOOR, SANITARY TRAYS AND CAGE WASHING MACHINE FILTERS.
(All eggs T. leonina)

ORIGIN	SAMPLE	TOTAL EGGS	L*	U*	D*
ROOM 1 PEN A (1)	FLOOR	6	5	1	0
ROOM 1 PEN A (2)	FLOOR	116	112	4	0
ROOM 1 PEN B (1)	FLOOR	14	13	1	0
ROOM 1 PEN B (2)	FLOOR	36	31	5	0
ROOM 6 PEN A	FLOOR	12	12	0	0
ROOM 6 PEN B	FLOOR	2	0	0	2
PENS	SAN. TRAY	3	0	3	0
PENS	SAN. TRAY	0	0	0	0
CAGES	SAN. TRAY	1	0	1	0
CAGES	SAN. TRAY	4	3	1	0
CAGE WASHING MACHINE	FILTER No. 1	13	10	3	0
CAGE WASHING MACHINE	FILTER No. 2	21	18	3	0
CAGE WASHING MACHINE	FILTER No. 3	6	6	0	0
CAGE WASHING MACHINE	FILTER No. 4	10	8	0	2

L* Larvated eggs
U* Unembryonated eggs
D* Degenerating eggs

Eggs/litre in floor and filter washings
Eggs/100 ml in sanitary tray washings

All the samples except one of the four sanitary tray washings contained ascarid eggs. However all samples were negative for coccidial oocysts. The mean number of eggs obtained from floor washings was greater than from the other sources and most of these eggs were larvated. From Room 1 Pen A (2) 96.5% of the eggs were in the infective stage, and from a total of 50 eggs present in the cage washing machine filters 84% were also larvated. The samples were taken from the sanitary trays immediately after leaving the cage washing machine and only a total of 8 eggs were recovered but 3 of these were infective.

3. TREATMENT OF CATS

Faeces from two queens was examined 24 hours after they were seen to have ingested all the food containing the dose of piperazine citrate and four and six worms were collected from them. Previous to treatment they had 2,300 and 3,500 eggs per gram in the faeces respectively.

All worms were identified as T. leonina. Faecal examinations carried out 7 and 14 days after treatment were negative for T. leonina eggs.

4. THE DEVELOPMENT OF T. LEONINA EGGS FROM DOMESTIC CATS

Egg development in artificial media in the laboratory and in the Centre for Laboratory Animals (C.L.A.) conditions are shown in Tables 11 and 12 and this data is illustrated in Graph 7.

TABLE 11. DEVELOPMENT OF T. LEONINA EGGS, FROM DOMESTIC CATS, IN ARTIFICIAL MEDIA IN THE LABORATORY

STAGE		DAYS OF INCUBATION							
		0	1	2	3	4	5	6	7
0	ONE CELL	100	12						
1	EARLY MORULA		22						
2	LATE MORULA		63	69	5	5	4	3	2
3	TADPOLE STAGE		3	30	24	1	2	3	3
4	MOTILE EMBRYO			1	71	94	94	94	95
TOTAL INDEX VALUE		0	157	232	366	389	390	391	393

TABLE 12. DEVELOPMENT OF T. LEONINA EGGS, FROM COMESTIC CATS, FROM FLOOR FAECAL SAMPLES IN THE C.L.A.

STAGE		DAYS OF INCUBATION							
		0	1	2	3	4	5	6	7
0	ONE CELL	100	57	65	35	22		9	
1	EARLY MORULA		42	16	16	15		10	
2	LATE MORULA		1	17	44	40		10	
3	TADPOLE STAGE			2	3	12		12	
4	MOTILE EMBRYO				2	11		59	100
TOTAL INDEX VALUE		0	44	56	121	175		302	400

The room temperatures in both cases were recorded daily and are shown in Table 13.

TABLE 13. ROOM TEMPERATURES IN °C FROM THE LABORATORY AND FROM THE C.L.A.

DAYS	LABORATORY		C.L.A.	
	MIN	MAX	MIN	MAX
1	19.0	24.5	18.0	22.5
2	20.0	26.5	18.0	23.0
3	20.0	27.0	18.0	23.0
4	19.5	27.5	18.5	23.0
5	20.0	31.5	-	-
6	20.0	28.5	17.5	21.0
7	19.0	27.5	18.0	21.0

The average temperature in the laboratory was 23.6°C and in the C.L.A. was 20.2°C.

Graph 7 shows that egg development in the artificial media was almost completed during the first three days of incubation and that 94% had developed within 4 days. Egg development in the floor faecal samples in the C.L.A. was slower than in the artificial media. Only 11% had fully developed at 4 days but all the eggs had reached this stage after 7 days.

5. ENZYME ELECTROPHORESIS

The zymograms are shown in Plates 15 and 16. Plate 15 shows that patterns for T. leonina larvae obtained from domestic cats (bands 1, 4, 8, 10) are different from a T. leonina adult obtained from a dog (band 3) and another from a domestic cat (band 5). The pattern for a T. canis adult obtained from a dog (band 2) differed from all the

T. leonina patterns.

A distinct difference between the patterns for T. leonina adults from dogs (bands 1,2) and a domestic cat (band 3) is shown in Plate 16. This zymogram also shows that the pattern for T. leonina larvae from domestic cats (band 4) is similar to that for a T. leonina adult obtained from a puma (band 5).

6. SEROLOGICAL TEST FOR TOXOPLASMA

None of the four cats tested revealed significant titres for Toxoplasma gondii.

7. FAECAL SAMPLES FROM KITTENS

The ten samples examined from rectal swabs collected from kittens between 8 and 12 weeks old were all negative for both ascarid eggs and coccidial oocysts.

PLATE 15

Zymogram of glucose phosphatase isomerase isoenzymes from extracts of T. leonina larvae and adults and T. canis adults on a thin layer starch gel.

Bands No:

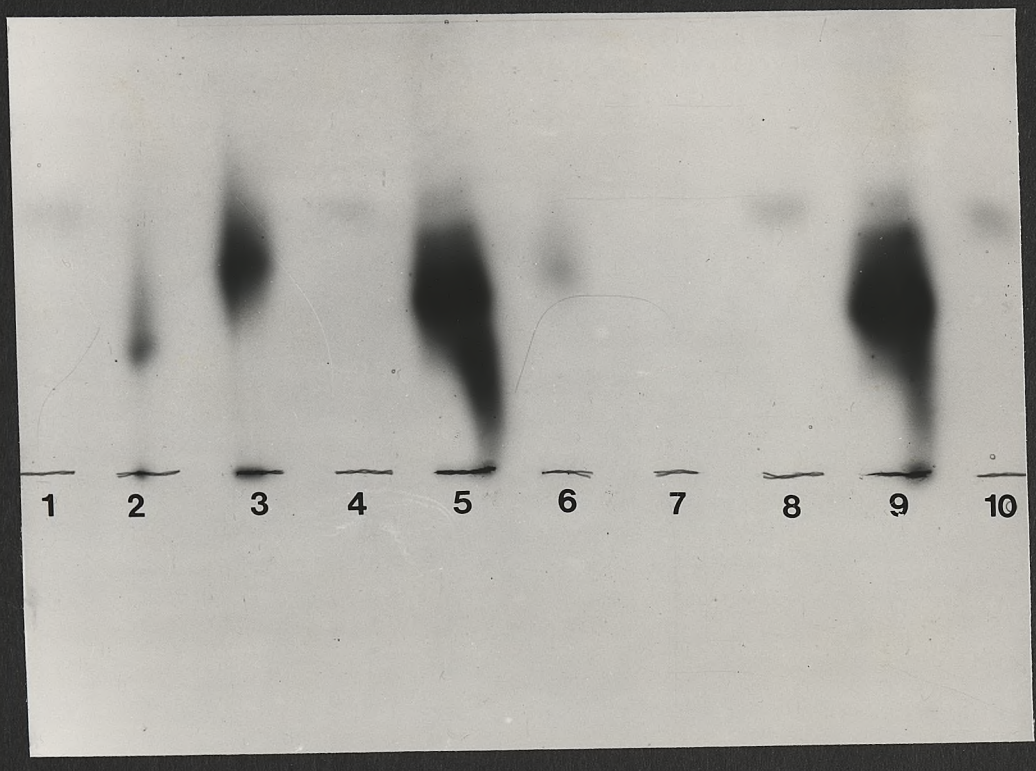
1. T. leonina larvae from domestic cats
2. T. canis adult from a dog
3. T. leonina adult from a dog
4. T. leonina larvae from domestic cats
5. T. leonina adult from a domestic cat
6. T. leonina adult from a dog
7. T. canis adult from a dog (not visible)
8. T. leonina larvae from domestic cats
9. T. leonina adult from a domestic cat
10. T. leonina larvae from domestic cats

PLATE 16

Zymogram of glucose phosphatase isomerase isoenzymes from extracts of T. leonina larvae and adults on a thin layer starch gel.

Bands No:

1 and 6 T. leonina adult from a dog
2 and 7 T. leonina adult from a dog
3 and 8 T. leonina adult from a domestic cat
4 and 9 T. leonina larvae from domestic cats
5 and 10 T. leonina adult from a puma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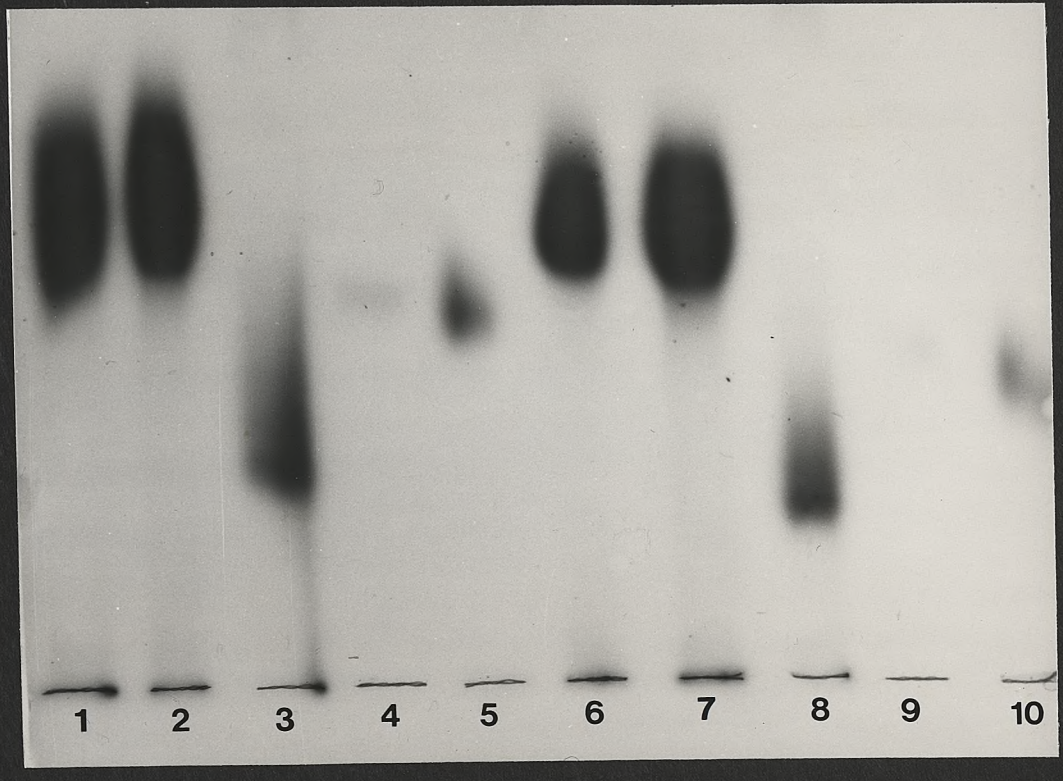
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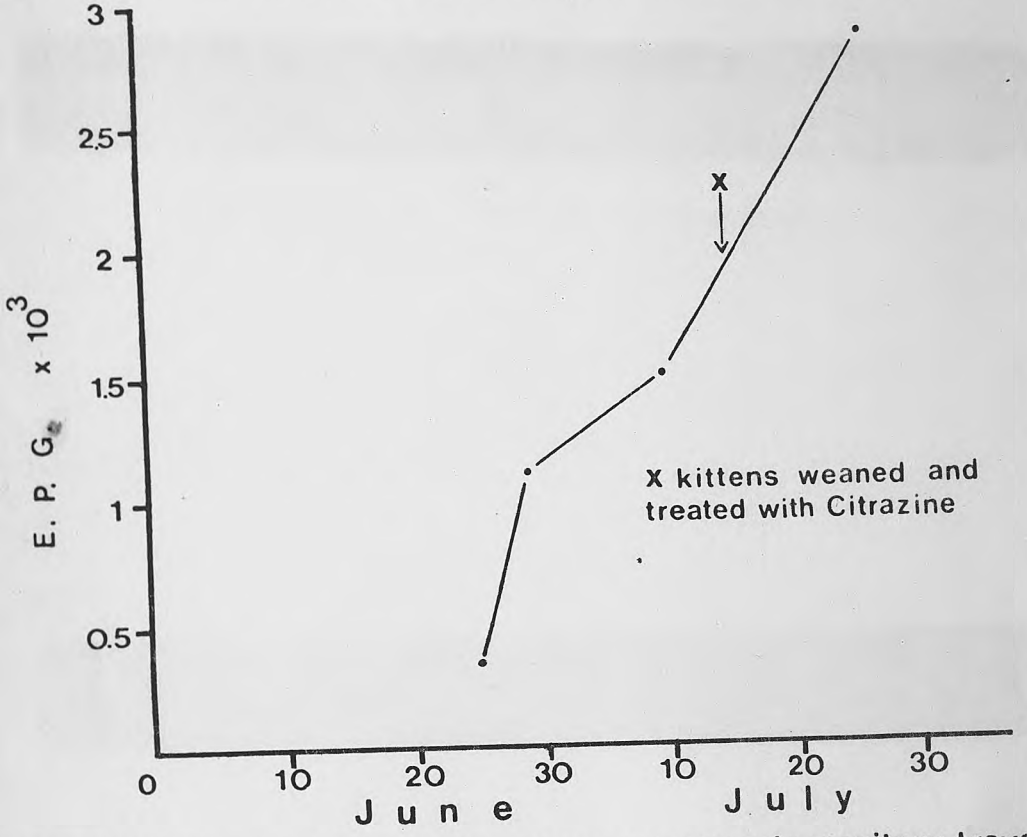
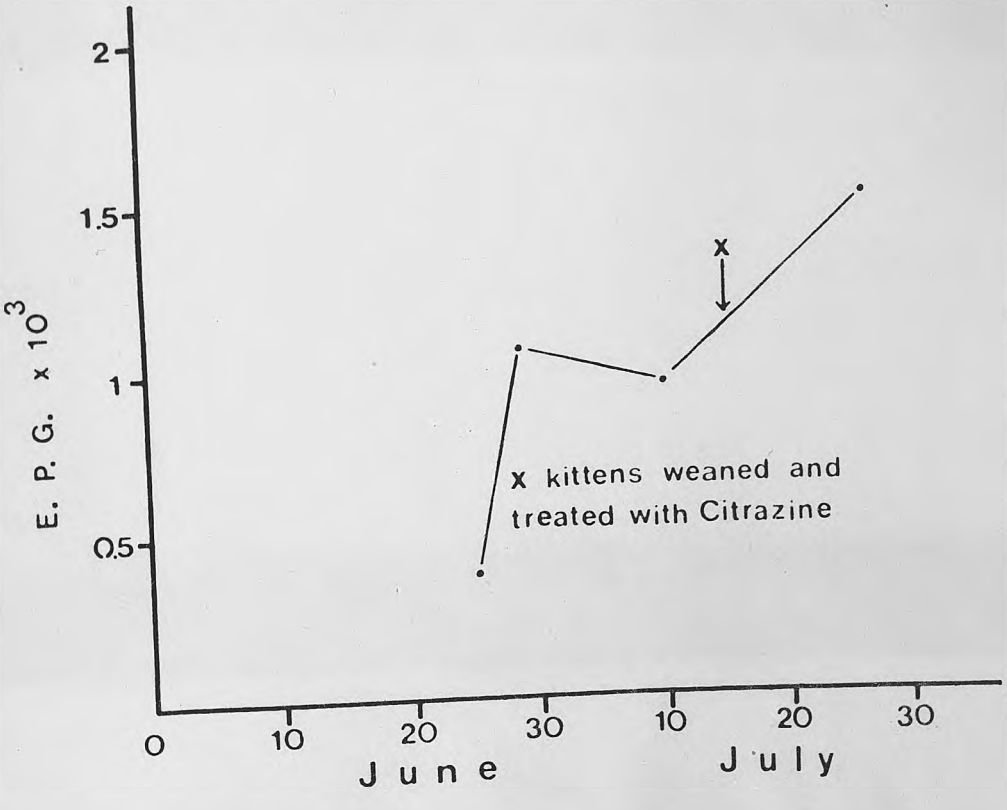
GRAPH 1 Faecal Toxascaris eggs per gram in sanitary trays in CAGE 14 from BLUE ROOM (kittens born on 15/5)

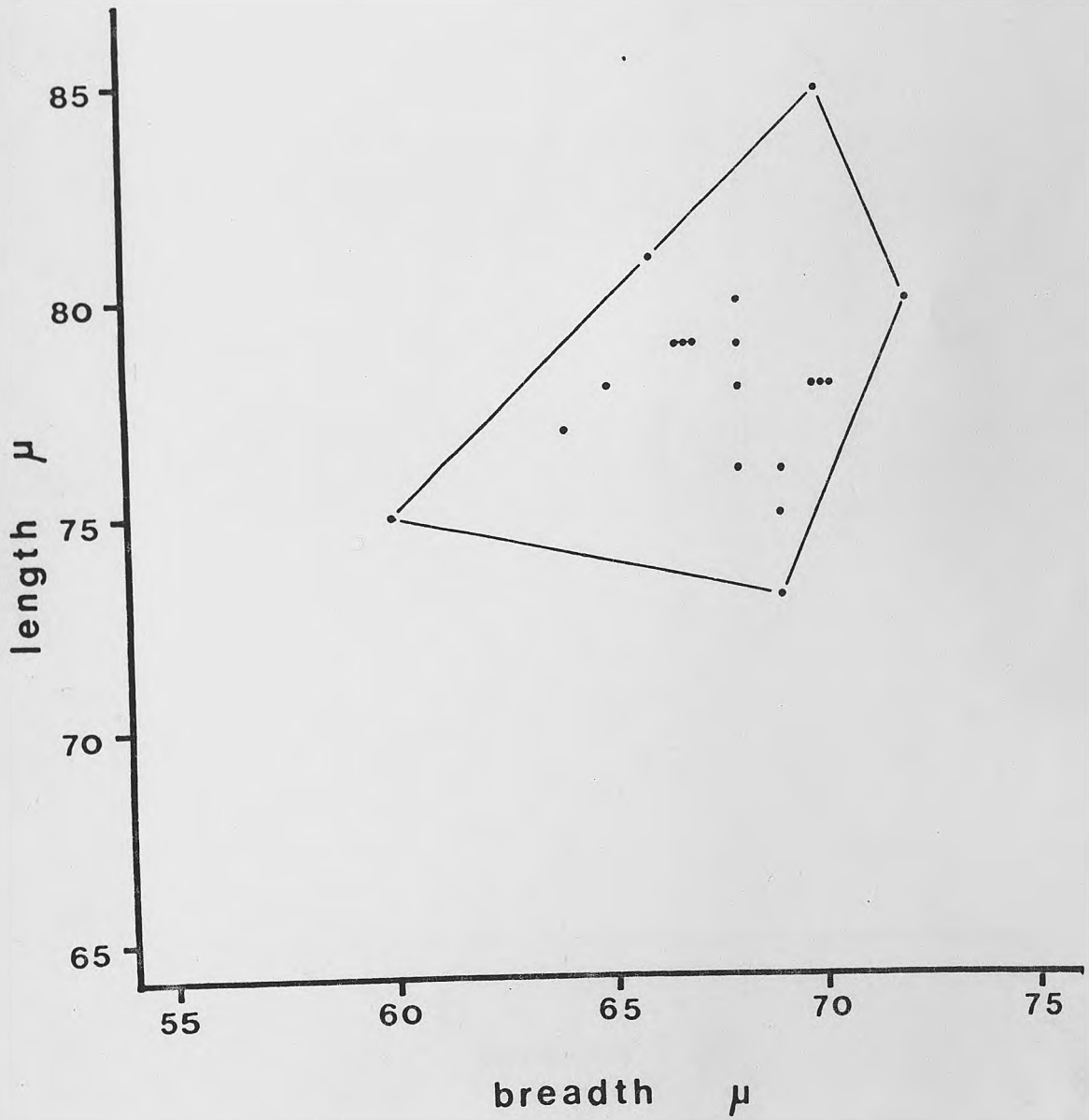


GRAPH 2 Faecal Toxascaris eggs per gram in sanitary trays in CAGE 4 from BLUE ROOM (kittens born on 17/5)



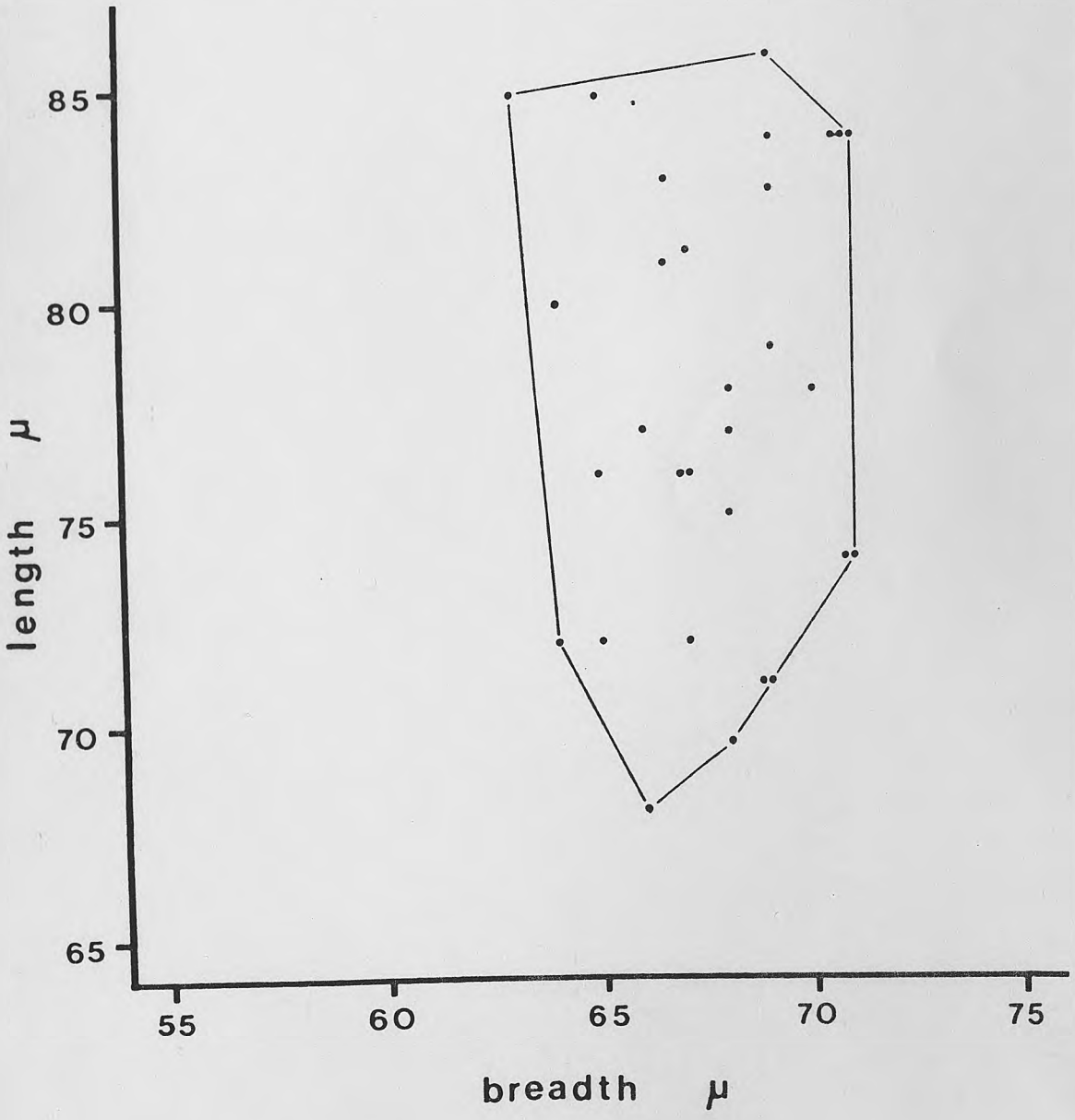
GRAPH 3 MINIMUM CONVEX POLYGON FOR

DOMESTIC CAT T. leonina EGGS

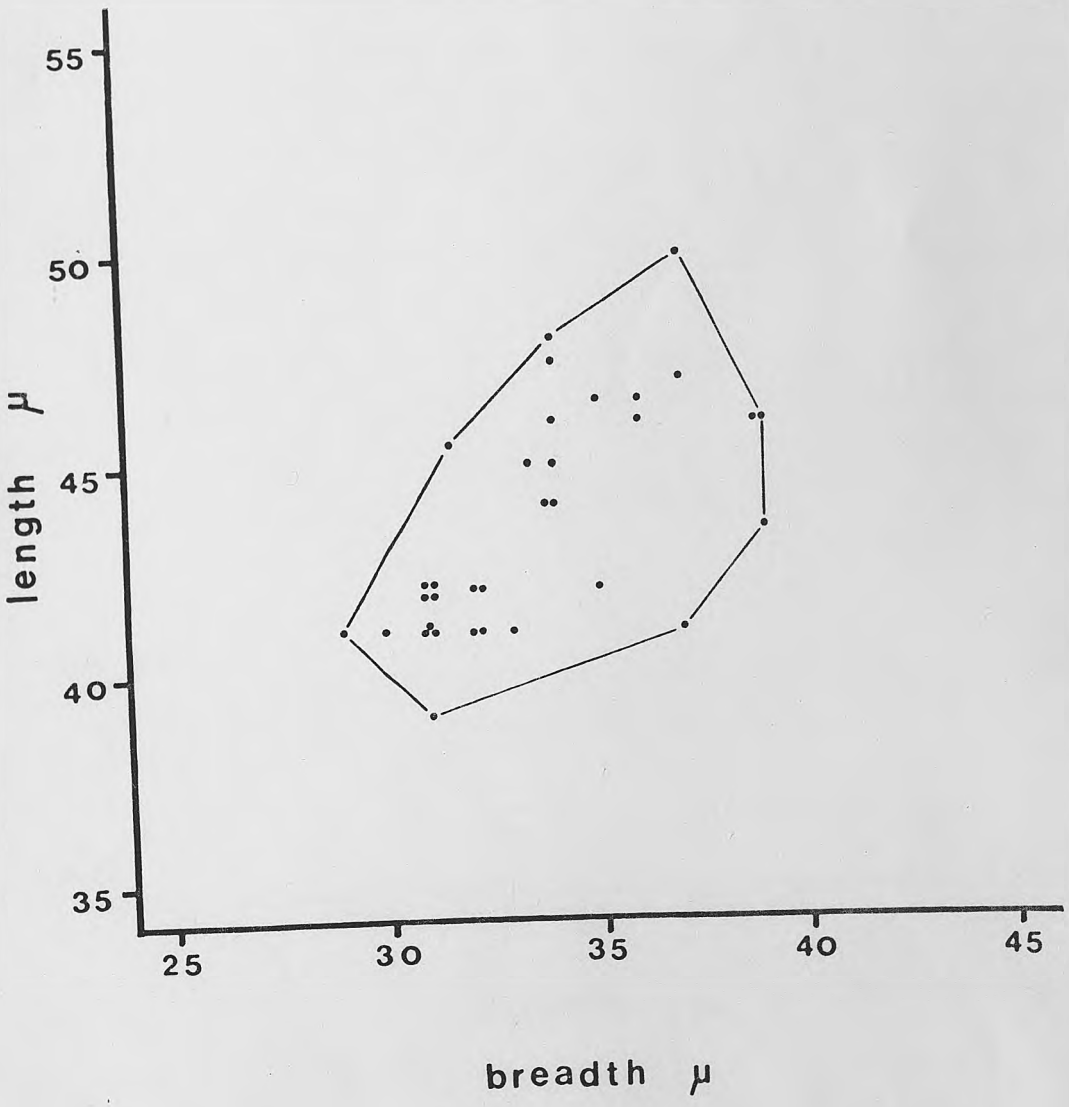


GRAPH 4 MINIMUM CONVEX POLYGON FOR

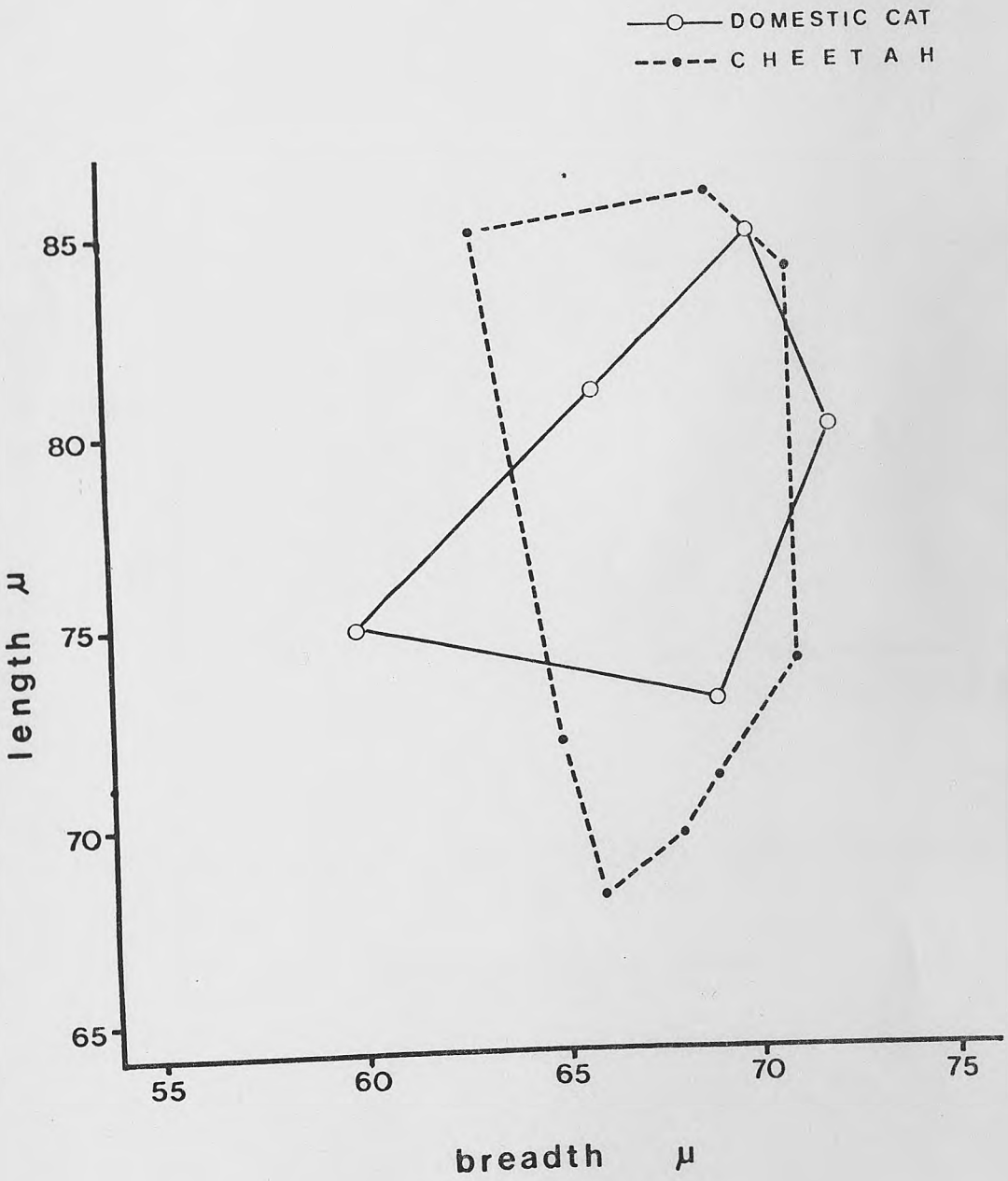
CHEETAH T. leonina EGGS



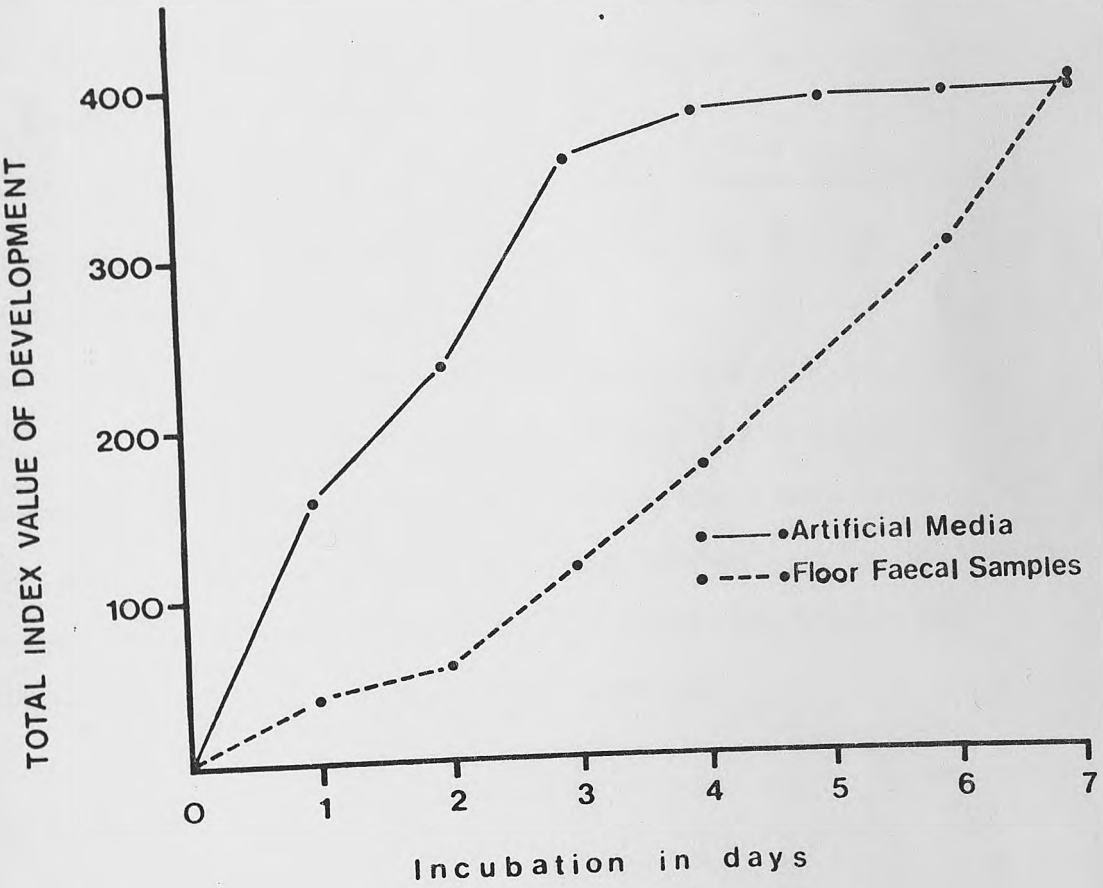
GRAPH 5 MINIMUM CONVEX POLYGON FOR DOMESTIC CAT *I. felis* OOCYSTS



GRAPH 6 T. leonina EGG PARAMETRES COMPARISON



GRAPH 7 DEVELOPMENT OF T.leonina EGGS FROM DOMESTIC CATS IN ARTIFICIAL MEDIA IN THE LAB. AND IN FLOOR FAECAL SAMPLES IN THE C.L.A.



DISCUSSION

The advent of highly efficient anthelmintics has played an increasing role in the elimination of helminth parasites. However a reduced prevalence may take place without the extensive use of drugs. Modern and hygienic methods of rearing and housing have proved highly efficient in reducing parasitism of animals in many countries.

Outbreaks of ascariasis and coccidiosis occur from time to time in kennels and catteries where large numbers of animals are kept together. Epidemiological studies have revealed that poor sanitation is often involved.

In the present study it was only possible to make observations for a period of seven months and a longer survey period would make the epidemiological picture more complete.

The sampling of faecal material presented several difficulties. Accurate identification of stool samples was not always possible particularly in the common pens where several animals were housed together. Regular collection of samples was dependant on the excellent co-operation of the staff of the Centre for Laboratory Animals and difficulties arose only in times of staff shortage.

FAECAL EXAMINATIONS

(i) Ascarid eggs. From a study of the literature, T. cati and T. leonina are parasites commonly found infecting Felidae particularly when kept in captivity. According to Keymer (1978), T. leonina is the only ascarid reported in Britain from captive Felidae. Garden (1978)

found at the Edinburgh Zoo that T. leonina was the predominant ascarid in the 'big cats' in captivity and T. cati was important in 'small cats'. The statement that T. cati is important in small cats is supported by Guterbock and Levine (1977) who found T. cati eggs in 32% and T. leonina in 6% of the faecal samples collected in a cat laboratory colony in the U.S.A.

Two out of three stray cats from the Cat and Dog Home autopsied by the author were also found to be infected with T. cati. Nevertheless the present study at the Centre for Laboratory Animals (C.L.A.) revealed that T. leonina was the only ascarid recovered from domestic cats. The colony has been a closed unit for several years and no reasonable conclusion was possible as to where and when Toxascaris infection was introduced.

Sosnowski (1973) considered that no age resistance occurs in Toxascaris infection in the Felidae and Visco et al (1978) concluded that ascarids are less prevalent in cats over 6 months of age. Although there are insufficient results from the present study to draw any conclusions about age resistance, the prevalence of T. leonina in adults was higher than in younger cats. As adult cats are housed in common pens harbouring several animals the reason for this higher infection amongst adults may be attributed to the fact that T. leonina is easily transmitted. This view is supported by Okoshi and Usui (1967a) who found that T. leonina infection is readily spread within a group of cats.

There were big differences in egg counts in the positive samples particularly in the faeces from adult cats (Table 4).

In spite of all animals being regularly treated with piperazine citrate, high egg counts were recorded in some samples. However no animals presented any clinical signs of ascariasis and all appeared to be healthy.

Eggs were recovered from the faeces of three animals which had been recently routinely treated. Sample 1/B (Table 4) and samples 14 and 4 (Graph 1,2) were positive 4 and 10 days after treatment respectively. This routine procedure of treating the cats in the C.L.A involves sprinkling piperazine citrate on the food which is then given to several cats in each pen. It is possible that problems might have arisen from this technique. It is well known that in a group of animals a 'pecking order' exists. Bullying animals and stronger ones usually get to the food first and eat the top layer on which the piperazine citrate has been sprinkled; thus that which is left for the others is likely to contain little or none. Furthermore any difference in taste or colour of the food caused by the anthelmintic may also tend to discourage some cats from eating it. It is concluded that an inadequate amount of the anthelmintic is ingested by some cats. This is seen in Plate 6 in kittens. It is suggested that this problem may be overcome by mixing the anthelmintic into the food.

Okoshi and Usui (1967c) found that immature T. leonina were more resistant to piperazine citrate than the mature worms. Consequently a subdose when associated with this lower efficiency of piperazine on immature worms may have been partly responsible for the maintenance of the infection.

Although it was only possible to make a few observations the piperazine citrate was effective in removing T. leonina when given to individual animals in the food at the recommended dose. The two cats

ingested all the treated food and in two subsequent faecal examinations no T. leonina eggs were recovered.

Garden (1978) found that piperazine adipate was less effective than the piperazine citrate. Nevertheless both compounds were considered unreliable in the treatment of ascariasis of wild Felidae when mixed in the food. The principal reason was that inadequate anthelmintic was ingested by the animals and eggs were often recovered after treatment. However Mann et al (1955) under experimental conditions found that piperazine citrate was highly effective in cats at a rate of 100 mg/kg.

Most of the samples were collected from sanitary trays where faeces and urine of several animals had become mixed together. Soft faeces and the fact that the cats liked to sit in the sanitary trays also contributed to this. Furthermore the faecal samples were invariably mixed with considerable quantities of wood shavings which were used as bedding. Thus it was almost impossible to obtain the same amount of faeces for all faecal egg counts.

A slight discrepancy existed between the results of the McMaster technique and the Saturated Salt Flotation technique. Although the McMaster technique presents considerable counting errors, results are adequate for clinical purposes (Sewell and Hammond, 1978, unpublished). Okoshi and Usui (1967c) concluded that the Saturated Salt Flotation technique was more sensitive than the McMaster technique. This was confirmed in the present study in that negative samples in the former were often positive in the latter.

(ii) Coccidial oocysts. Coccidiosis has often been reported in the cat. In spite of the fact that 13% of the faecal samples examined harboured Isospora felis oocysts, only in one occasion (BLUE/6, Table 7) large numbers were recovered. Although the animals were never treated, the only clinical sign was a very mild diarrhoea in a few animals.

It seems that I. felis is not a serious threat to cats. Pellérdy (1965) attributes it to the subepithelial endogenous development of the parasite and that only after exceptionally massive infestation can it cause acute haemorrhagic enteritis.

Soulsby (1978) stressed that the condition 'coccidiosis' based on clinical signs and the presence of large numbers of oocysts in the faeces should be taken with care as kittens are prone to various enteritides due to other causes which may release oocysts from the subepithelial tissues.

Most of the positive samples were recovered from individual cages containing kittens only. This fact is in accordance with the observations of several authors in that young animals are more susceptible to coccidial protozoa. Giorgi (1969) concluded that after an initial subpathogenic challenge the host acquires a certain degree of resistance and becomes free of infection or a carrier. Oocysts were found in two faecal samples from adults (Table 7) and it is possible that these animals were carriers.

Toxoplasmosis has become increasingly important as a zoonosis and the cat represents an important vector in the transmission of T. gondii to man (Peterson, Tronca and Bonin, 1972). Small oocysts, particularly those of I. bigemina are almost indistinguishable from Toxoplasma oocysts. However the oocysts of I. felis are very much

larger than those of T. gondii. Dubey (1976) found that cats chronically infected with T. gondii acquire immunity and rarely shed oocysts in the faeces but suggests that I. felis infection may reactivate a latent toxoplasma infection by interfering with local immunity. Because of these findings it was decided to perform serological tests for toxoplasmosis in some of the cats shedding large numbers of I. felis oocysts. The four cats tested were negative for Toxoplasma antibodies but greater numbers of animals should be tested before a definite conclusion on the presence or absence of T. gondii in the colony can be reached.

The observation that no clinical signs were seen is in accordance with the general view that I. felis infection is benign and subclinical in adult cats.

Wilkinson (1977) concluded that eradication of I. felis from a cat colony can only be achieved if food and water are provided in disposable dishes and discarded after each meal and he considered that sulphadimethoxine produced a reasonably rapid elimination of oocysts from faeces.

MEASUREMENTS OF EGGS AND OOCYSTS

Significant differences in the sizes of T. leonina eggs from several host species are described in the literature. Okoshi and Usui (1967b) found that measurements of T. leonina eggs from dogs, cats, tigers and cheetahs differed considerably. The largest differences were between the canine and the feline strain. Garden (1978) also found that a disparity existed between T. leonina eggs of the dog

and Felidae and that the mean egg parameters were lower than those recorded by Okoshi and Usui (1967b). The difference was possible due to the fact that the former measurements were made on unembryonated eggs whereas the latter were made on fully embryonated eggs.

Results of the measurements of T. leonina eggs from domestic cats and the cheetah in the present study showed that no differences existed. This is illustrated in Graph 6.

The present measurements were made in fully embryonated eggs and the mean egg parameters for T. leonina eggs from domestic cats were similar to those found by Okoshi and Usui (1967b).

Some discrepancies exist in the literature about the sizes of I. bigemina oocysts. However with I. felis this does not occur and the results of measurements and studies on the sporulation of the oocysts in the present work are in accordance with the findings of several authors (Levine, 1961; Pellérdy, 1965 and Wenyon, 1965).

EXAMINATION OF FLOOR, SANITARY TRAY AND FILTER WASHINGS

The technique used for the examination of the suspensions of the different washings was reliable but the eggs were found to be collapsed when left in the suspensions for several hours at 4°C. This made examination difficult and the problem was overcome by examining fresh suspensions.

A quantitative comparison of eggs from different sources was impossible. Although lower numbers of eggs were recovered from washings from the sanitary trays than from the floor all should have been removed from the sanitary trays in the cage washing machine. Further-

more some of the eggs which were removed from the machine filters were larvated. The critical development temperature for T. leonina eggs from domestic cats is 37°C (Okoshi and Usui, 1968b). When in operation the temperature in the cage washing machine reaches 90°C but all the eggs were not instantly destroyed. A longer period of time than the 2 min 45 sec for which the sanitary trays are in the machine may be necessary to destroy the eggs.

Better results might be achieved by autoclaving the cages and sanitary trays. However this is not possible at the C.L.A. as the autoclave is not big enough and moreover it is fully utilized for the cages and sanitary trays from the minimal disease unit of the Centre.

In suitable conditions of temperature (15-35°C) T. leonina eggs reach the infective stage in 3-6 days (Soulsby, 1965). The average room temperature recorded from the C.L.A. (20.2°C) during July 1979 was within this range and the fact that romms are cleaned at two week intervals may explain why large numbers of larvated eggs were recovered from the floor.

DEVELOPMENT OF T. LEONINA EGGS

In both the experiments almost all T. leonina eggs from domestic cats developed to the infective stage within seven days. Egg development in the artificial media was quicker than in the floor faecal samples particularly during the first four days. This may be due to the fact that the average temperature of the laboratory (23.6°C) was higher than the C.L.A. (20.2°C). These results support those of Okoshi and Usui (1968b) who found that T. leonina eggs from felines when incubated

at 25°C developed quicker than between 17-22°C during the first four days.

During winter the whole building at the C.L.A. is centrally heated and the average temperature of the nursery rooms is about 17.7°C and that of the pens is slightly lower. According to Okoshi and Usui (1968b) these are within the optimal temperature range for development of the eggs.

ENZYME ELECTROPHORESIS

Experiments using the enzyme electrophoresis technique indicated that larvae obtained from fully embryonated eggs can be used when adult worms are unobtainable. The weak mobility bands in both zymograms (Plates 15, 16) was possibly due to overdilution of the extracts or lack of enzyme. Although in Plate 16 samples 6 to 10 are replicates of samples 1 to 5, there is a slight difference in the mobility bands particularly between samples 3 and 8 and between samples 5 and 10. This distortion may have been caused by an uneven starch gel or an imperfect adjustment of the plate between the electrodes.

Several studies suggested that there is a difference between canine and feline strains of T. leonina (Sprent, 1959, and Okoshi and Usui, 1967b). The results of the isoenzyme analysis using the enzyme glucose phosphatase isomerase are in accordance with the results of Garden (1978) who found that the mobility band for T. leonina from the dog was different from the bands from the Felidae. Differences in the mobility bands were also found between T. leonina larvae from domestic cats and adult worms from the same host species. It was impossible to extend these studies because of the time factor.

SUGGESTIONS

The three main suggestions for the control of ascarid infections at the C.L.A. are:

1. The anthelmintic should be well mixed into the food instead of sprinkling it on the top. Possibly the blender which is used in the preparation of the food could be used for this purpose. The treated food should then be fed ad lib.

2. As most of the T. leonina eggs become larvated within seven days cleaning intervals should be less than this to be effective.

3. The cleaning methods in present use should be further evaluated.

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REFERENCES

ADAM, K.M.G., PAUL, J. & ZAMAN, V. (1971). Medical and Veterinary Protozoology, p. 167. Edinburgh. London: Churchill Livingstone.

ALEXANDER, R. (1976). An Introduction to Veterinary Pharmacology, 3rd ed. pp. 324-334. Edinburgh. London. New York: Churchill Livingstone.

ANTONENKO, A.E. (1976). Survival of helminth eggs and coccidial oocysts of swine in litter-free liquid manure. Dostizheniya Veterinaroi Nauki i Peredovogo Opyta-zhivotnovodstvu, 2, 24-27. Cited from Veterinary Bulletin, Weybridge. (1977). 47, abstract 5066.

BEAVER, P.C. (1956). Larva migrans. Experimental Parasitology, 5, 587-621.

BEAVER, P.C. (1969). The nature of visceral larva migrans. Journal of Parasitology, 55, 3.

BURROWS, R.B. & LILLIS, W.C. (1962). Stilbazium iodide as an anthelmintic. II. Against various nematodes of dogs and cats. Journal of Parasitology, 48, 769-771.

BROWN, H.W. (1928). Further studies on the longevity of the eggs of Ascaris lumbricoides and A. suum. Journal of Parasitology, 15, 14-21.

CAMPANA-ROUGET, Y., DORCHIES, P. & GOURDON, L. (1974). Reciprocal effects of Toxoplasma and Isospora in their development in the cat. Comptes Rendus des Scéances de la Societé de Biologie, 168, 514-516. Cited from the Veterinary Bulletin, Weybridge. (1975). 45, abstract 1775.

CHESSUM, B.S. (1972). Reactivation of Toxoplasma oocysts production in the cat by infection with Isospora felis. British Veterinary Journal, 128, xxxiii-xxxvi.

CLEELAND, R. & LAURENCE, K.A. (1962). In vitro cultivation of Ascaris lumbricoides var. suum larvae. Journal of Parasitology, 48, 35-38.

DODSON, C., CAMPBELL, R.W. & WEBB, A.I. (1967). Anaphylactic and Reagin-like antibody in rats infected with Toxocara canis larvae. Journal of Parasitology, 53, 209.

DOUGLAS, J.R. & BAKER, N.F. (1965). Some Host-Parasite Relationships of Canine Helminths. In: Host-Parasite Relationships (Proceedings of the 26th Annual Biology Colloquium, April 23-24, 1965). Edited by James E. McCauley, pp. 97-115. Oregon State Univ. Press.

DUBEY, J.P. & FRENKEL, J.K. (1972). Extra-intestinal stages of Isospora felis and I. rivolta (Protozoa: Eimeriidae) in cats. Journal of Protozoology, 19, 89-92.

DUBEY, J.P. (1976). Reshedding of T. gondii oocysts by chronically infected cats. Nature, U.K., 262, 213-214.

DUBEY, J.P. & STRIETEL, R.H. (1976). Isospora felis and I. rivolta infections in cats induced by mouse tissue or oocysts. British Veterinary Journal, 132, 649-651.

DUNN, A.M. (1969). Veterinary Helminthology. London: William Heinemann Medical Books Ltd.

EL HINDAWY, M.R. (1948). The studies on the blood of dogs - II. Haematological findings in (A) apparently healthy dogs harbouring intestinal parasites; (B) dogs infested with Spirocerca sanguinolenta. Veterinary Journal, 104, 159-165.

EL MOUKDAD, A.R. (1976). Effect of disinfectants on preparasitic development stages. I. Effect on Eimeria oocysts. Weiner Tierärztliche Monatsschrift, 63, 399-405. Cited from Veterinary Bulletin, Weybridge. (1977). 47, abstract 5340.

EL MOUKDAD, A.R. (1977). Effect of several disinfectants on preparasitic developmental stages. II. Effect on eggs of Ascaris suum and Toxascaris leonina. Weiner Tierärztliche Monatsschrift, 64, 85-91. Cited from the Veterinary Bulletin, Weybridge. (1977). 47, abstract 5341.

FAIRBAIRN, D. (1961). The in vitro hatching of Ascaris lumbricoides eggs. Canadian Journal of Zoology, 39, 153-162.

GARDEN, N.J. (1978). Epidemiology of Ascariasis in Felidae at Edinburgh Zoological Park. Master of Science thesis in the University of Edinburgh.

GIORGI, J.R. (1969). Parasitology for Veterinarians, pp. 8-128. Philadelphia. London. Toronto: W.B. Saunders Company.

GIRDWOOD, R.W.A., QUINN, R., BRUCE, R.G. & SMITH,H.V. (1976). Incidence of Toxocara and Toxascaris in Glasgow. Transactions of the Royal Society of Tropical Medicine and Hygiene, 70, 284.

GLICKMAN, L.T. & CYPESS, R.H. (1977). Toxocara infection in animal hospital employees. American Journal of Public Health, 67, 1193-1195.

GREENBERG, J., SEYMOUR, W.E. & McEWEN, A.F. (1958). Effect of varying doses of piperazine citrate on puppies. Veterinary Medicine, 53, 609-610.

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.

HITCHCOCK, D.J. (1955). The life-cycle of Isospora felis in the kitten. Journal of Parasitology, 41, 383-393.

HOGARTH-SCOTT, R.S. (1967). The molecular weight range of nematode allergens. Immunology, 13, 535-537.

HOGARTH-SCOTT, R.S., JOHANSSON, S.G.O. & BENNICH, H. (1969). Antibodies to Toxocara in the sera of visceral larva migrans patients: The significance of raised levels of IgE. Clinical and Experimental Immunology, 5, 619-625.

HUTCHINSON, W.M., DUNACHIE, J.F., WORK, K. & SIIM, CHR. J. (1971). Life cycle of the coccidian parasites, Toxoplasma gondii in the domestic cat. Transactions of the Royal Society of Tropical Medicine and Hygiene, 65, 380-399.

ITO, S., TSUNODA, K., NISHIKAWA, H. & MATSUI, T. (1974). Small type of Isospora bigemina: isolation from naturally infected cats and relation with Toxoplasma oocysts. National Institute of Animal Health, Japan, 14, 137-144. Cited from Veterinary Bulletin, Weybridge. (1975). 45, abstract 712.

JACOBS, D.E. & PEGG, E.J. (1976). Gastro-intestinal nematodes of élite show dogs in Great Britain. Journal of Helminthology, 50, 265-266.

JACOBS, D.E. & PROLE, J.H.B. (1976). Helminth infections of British dogs: prevalence in race grey-hounds. Veterinary Parasitology, 1, 377-387.

KEYMER, I.F. (1978). Report of the Pathologist. In: The Zoological Society of London Scientific Report 1975-1977. Journal of Zoology, 184, 300.

KIRK, R.W. (1977). Current Veterinary Therapy. VI. Small Animal Practice, pp. 964-965. Philadelphia. London. Toronto: W.B. Saunders Company.

LE RICHE, P.D. & SEWELL, M.M.H. (1977). Differentiation of Taenia saginata and Taenia solium by enzyme electrophoresis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 71, 327-328.

LEVINE, N.D. (1961). Protozoan Parasites of Domestic Animals and of Man, pp. 238-242. Minneapolis: Burgess Publishing Company.

LEVINE, N.D. (1968). Nematode Parasites of Domestic Animals and of Man, pp. 344-354. Minneapolis: Burgess Publishing Company.

MANN, P.H., HARFENIST, M. & de BEER, E.J. (1955). The effectiveness of piperazine citrate against intestinal helminth of the cat and dog. Journal of parasitology, 41, 575-578.

MATTHEWS, R.E.F. (1977). Minimum Convex Polygons for the Deliniation of of Possible Viral Taxa. Journal of General Virology, 36, 41-50.

MERZ-SCHENKER, R.C., FREUDIGER, V. & HORNING, B. (1976). Clinical and public health importance of gastrointestinal parasites (helminth and protozoa) in the domestic cat. Kleintierpraxis, 21, 141-146. Cited from Veterinary Bulletin, Weybridge. (1977). 47, abstract 329.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. (1977). Manual of Veterinary Parasitological Laboratory Techniques, Technical Bulletin No. 18, Her Majesty's Stationery Office, London.

OKOSHI, S. & USUI, M. (1967a). Experimental Studies on Toxascaris leonina. I. Incindence 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 Toxascaris leonina. III. Morphology of worms and eggs obtained from various animals. Japanese Journal of Veterinary Science, 29, 329-336.

OKOSHI, S. & USUI, M. (1967c). Experimental studies on Toxascaris leonina. II. Diagnosis and treatment of Toxascariasis in dogs and cats. Japanese Journal of Veterinary Science, 29, 245-250.

OKOSHI, S. & USUI, M. (1968a). Experimental studies on Toxascaris leonina. V. Experimental infection of dogs and cats with eggs of canine, feline and Felidae strains. Japanese Journal of Veterinary Science, 30, 81-91.

OKOSHI,S. & USUI, M. (1968b). Experimental studies on Toxascaris leonina. IV. Development of eggs of three Ascarids, T. leonina, T. cati and T. canis in dogs and cats. Japanese Journal of Veterinary Science, 30, 29-38.

OKOSHI, S. & USUI, M. (1968c). Experimental studies on Toxascaris leonina. VI. Experimental infection of mice, chickens and earthworms with T. leonina, T. canis and T. cati. Japanese Journal of Veterinary Science, 30, 151-166.

PEGG, E.J. (1977). A new approach to the control of Toxocara canis and other parasitic ova on concrete-floored kennel runs. British Veterinary Journal, 133, 427-431.

PELLÉRDY, L. (1965). Coccidia and Coccidiosis, pp. 471-479. Budapest: Akadémiai Kiadó.

PETERSON, D.R., TRONCA, E. & BONIN, P. (1972). Human Toxaplasmosis. Prevalence and exposure to cats. American Journal of Epidemiology, 96, 215-218.

PITTS, T.D. (1963). In vitro cultivation of newly hatched larvae of Ascaris from man and swine. Journal of Parasitology, 49, 1034-1035.

ROGERS, W.P. (1960). The physiology of infective processes of Nematode parasites; the stimulus from the animal host. Proceedings of the Royal Society, London, 152, 367-386.

RONÉUS, O. (1971). Studies on the Inter-relationship between the Number of Orally Administered A. suum Eggs, Blood Eosinophilia and the Number of Adult Intestinal Ascarids. Pathology of Parasitic Diseases. Edited by S.M. GAAFAR, pp. 339-343. Purdue University Studies: Lafayette, Indiana.

SCUPIN, E., BRANDES, B., NIESSEN, C. & SCUPIN, Erica (1976). Use of Pyrantel pamoate paste and its efficacy against naturally occurring ascariasis. Kleinter Praxis, 216, 224-226. Cited from the Veterinary Bulletin, Weybridge. (1977). 47, abstract 1010.

SEWELL, M.M.H. & HAMMOND, J.A. (1978). Helminthological Techniques, unpublished.

SEWELL, M.M.H. & URQUHART, H.R. (1976). The recovery of helminth eggs from soil samples. Transactions of the Royal Society of Tropical Medicine and Hygiene, 70, 287.

SEWELL, M.M.H. (1979). Personal communication.

SOSNOWSKI, A. (1973). Parasitische Nematoden bei wilden Feliden. Zoologische Garten, 43, 204-209. In: GARDEN, N.J. (1978) Epidemiology of Ascarids in Felidae at Edinburgh Zoological Park. Master of Science thesis in the University of Edinburgh.(Original not seen).

SOULSBY, E.J.L. (1965). Textbook of Veterinary Clinical Parasitology. Vol.I, Helminths, pp. 8-52. Oxford: Blackwell Scientific Publications.

SOULSBY, E.J.L. (1978). Helminths, Arthropods and Protozoa of Domesticated Animals. (6th Edition of Monnig's Veterinary Helminthology and Entomology), pp. 160-168. London: Baillière, Tindall and Cassel.

SPRENT, J.F.A. (1952). On the migratory behaviour of the larvae of various Ascaris species in white mice. 1. Distribution of larvae in tissues. Journal of Infectious Diseases, 90, 165-176.

SPRENT, J.F.A. (1956). The life history and development of Toxocara cati (SCHRANK 1788) in the domestic cat. Parasitology, 46, 54-57.

SPRENT, J.F.A. (1958). Observations 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. (1962). The Evolution of the Ascaridoidea. Journal of Parasitology, 48, 818-824.

SPRENT, J.F.A. (1968). Notes on Ascaris and Toxascaris, with a definition of Baylisascaris gen nov. Parasitology, 58, 185-198.

STEDE, G. (1971). Desinfektionsveruche mit lysococ an kokzidienoozysten und askarideneiern. Dissertation, Berlin, p. 22. (In German). Cited from Helminthological Abstracts. (1971). 40, abstract 5058.

STEVENSON, P. & JACOBS, D.E. (1977). Toxocara infections in pigs. The use of indirect fluorescent antibody tests and an in vitro larval precipitate test for detecting specific antibodies. Journal of Helminthology, 51, 149-154. Cited from Helminthological Abstracts. (1977). 46, abstract 4854.

STEWART, J.S. (1947). Coccidiosis in the dog: Dosage of sulphamezathine in relation to its potential use. The Veterinary Record, 59, 378.

THORBURN, H. & WILLIAM, H. (1972). A stable haemagglutinating antigen for detecting Toxoplasma antibodies. Journal of Clinical Pathology, 25, 762-767.

VASILEF, I. (1961). Acquired immunity against Toxascaris leonina in dogs. I. Immunity to superinfection. Izv. tseut. Khelmint. Lab. Sof. 6, 5-19. (In Bulgarian). Cited from Helminthological Abstracts. (1968). 37, abstract 1908.

VIENS, P., STRYKOWSKI, H., RICHARDS, R. & SONEA, S. (1975). A modified immunofluorescent antibody technique for the serodiagnosis of human toxocaral larva migrans. Canadian Journal of Public Health, 66, 237-240. From Tropical Disease Bulletin, 73, 100. Cited from Helminthological Abstracts. (1976). 45, abstract 5737.

VISCO, R.J., CORWIN, R.M. & SELBY, L.A. (1978). Effect of age and sex on the prevalence of intestinal parasitism in cats. Journal of the American Veterinary Medical Association, 172, 797-800.

VON BRANDT, T. (1973). Biochemistry of Parasites, pp. 350-363. New York. London: Academic Press.

WENYON, C.M. (1965). Protozoology. Vol. II. (facsimile of 1926 edition), A Manual for Medical Men, Veterinarians and Zoologists, pp. 808-818. London: Baillière, Tindall and Cassel.

WILKINSON, G.T. (1977). Coccidial infection in a cat colony. The Veterinary Record, 100, 156-157.

WOODRUFF, A.W. (1976). Toxocariasis as a Public Health Problem. Environmental Health. London School of Hygiene and Tropical Medicine, 84, 29-31. Cited from Helminthological Abstracts. (1976). 45, abstract 4246.

WORLD HEALTH ORGANISATION TECHNICAL REPORT SERIES. No. 277. (1964). Soil-Transmitted Helminths. Report of a W.H.O. Expert Committee on Helminthiasis.

WORLD HEALTH ORGANISATION TECHNICAL REPORT SERIES. No. 379. (1967). Control of Ascariasis. Report of a W.H.O. Expert Committee.

WRIGHT, W.H. (1935). The relation of Vit. A. deficiency to Ascariasis in the dog. Journal of Parasitology, 21, 433.

YAMAGUTI, S. (1961). Systema Helminthum, Vol.3. The Nematodes of Vertebrates, part I. London. New York: Interscience Publishers.

ZEE, S.D. & ZINKHAM, W.H. (1975). Demonstration of parasitism in tissues by electrophoretic definition of parasite enzymes in host tissues. The Journal of Pediatrics, 86, 408-410.

EGG PARAMETERS OF T. LEONINA

	TABLE I - DOMESTIC CATS			TABLE II - CHEETAH		
	LENGTH um	x	BREADTH um	LENGTH um	x	BREADTH um
1.	79.0		67.0	72.0		67.0
2.	78.0		70.0	83.0		66.5
3.	80.0		68.0	76.0		65.0
4.	78.0		70.0	77.0		68.0
5.	80.0		72.0	75.0		68.0
6.	76.0		69.0	78.0		68.0
7.	75.0		60.0	72.0		64.0
8.	78.0		70.0	84.0		71.0
9.	77.0		64.0	76.0		67.0
10.	85.0		70.0	74.0		71.0
11.	73.0		69.0	71.0		69.0
12.	76.0		68.0	84.0		71.0
13.	78.0		70.0	76.0		67.0
14.	79.0		67.0	74.0		71.0
15.	75.0		69.0	71.0		69.0
16.	78.0		65.0	84.0		69.0
17.	79.0		68.0	85.0		63.0
18.	81.0		66.0	81.0		66.5
19.	79.0		67.0	81.5		67.0
20.	78.0		68.0	70.0		67.0
21.				72.0		65.0
22.				80.0		64.0
23.				86.0		69.0
24.				83.0		69.0
25.				79.0		69.0
26.				78.0		70.0
27.				85.0		65.0
28.				68.0		66.0
29.				84.0		71.0
30.				77.0		76.0

OOCYST PARAMETERS OF I. FELIS

TABLE III - DOMESTIC CATS

	LENGTH um	x	BREADTH um
1.	42.0		35.0
2.	46.5		36.0
3.	41.0		29.0
4.	50.0		37.0
5.	41.0		33.0
6.	44.0		34.0
7.	46.0		36.0
8.	44.0		34.0
9.	46.0		34.0
10.	42.0		31.0
11.	46.5		35.0
12.	41.0		31.0
13.	39.0		31.0
14.	41.0		37.0
15.	43.5		39.0
16.	42.0		32.0
17.	41.0		32.0
18.	45.0		33.5
19.	42.0		32.0
20.	46.0		39.0
21.	42.0		31.0
22.	41.0		32.0
23.	45.0		34.0
24.	42.0		31.0
25.	41.0		31.0
26.	48.0		34.0
27.	42.0		31.0
28.	47.0		37.0
29.	47.5		34.0
30.	41.0		31.0