INVESTIGATION OF THE INVOLVEMENT OF THE DOMESTIC CAT IN THE CYCLIC TRANSMISSION OF CAPRINE BESNOITIA

BY

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DECLARATION

I hereby declare that this thesis is my original work except where acknowledged and has not been presented in any other University.

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DEDICATION

DEDICATED TO

MY FATHER AND MOTHER

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ABSTRACT

This study covers various aspects of coccidia of the domestic cat under natural and experimental conditions and the cat's involvement in the cyclic transmission of caprine *Besnoitia*.

In a preliminary study on natural coccidian infection in cats around Kabete, fifty faecal samples examined revealed oocysts of four of the five known genera, *Isospora*, *Sarcocystis*, *Toxoplasma* and *Besnoitia*. Mixed and monospecific infections were observed in 46% and 38% of the samples respectively, while 16% had no infection. Oocysts of *Isospora felis* were the largest and measured on average 42.4 by 34.0 μm (n = 50). They were also the most frequent (66%) followed by *Isospora rivolta* (44%). Oocysts of *I. rivolta* measured 25.2 by 23.2 μm on average (n = 50). Free sporocysts of *Sarcocystis muris* were recovered in 4% of the samples and measured on average 11.0 by 7.9 μm (n = 50). *Toxoplasma gondii* oocysts were found in 12% of the samples and measured on average 12.0 by 10.3 μm (n = 50). *Besnoitia wallacei* oocysts were recovered in cat faeces for the first time in Kenya and occurred in 4% of the samples. They measured on average 16.6 by 14.5 μm (n = 50).

Rats, mice and rabbits orally infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts developed the characteristic tissue cysts. *Toxoplasma* cysts in the brain of mice were spherical and measured 22.9 by 18.6 μm on average (n = 20). Spherical to ovoid cysts of *Besnoitia wallacei*, with the characteristic thick and PAS positive wall were seen in tissues of rats, mice and rabbits. In mice they measured 208.5 by 164.1 μm on average (n = 25). This observation indicated that *Toxoplasma* and *Besnoitia* are present around Kabete.

Endogenous stages of *Besnoitia wallacei* recovered from one of the cat faecal sample were studied in 13 other cats. The cats were fed on infected mice and rat tissues and serially sacrificed at one to two day intervals for 16 days. Asexual and sexual stages were seen between day 6 and 16 of infection. Microschizonts measuring 22.6 by 14.7 μm (n = 2) were seen in epithelial cells of the small intestines while macroschizonts occurred in the lamina propria of the small intestines and the liver during this period. Macroschizonts in the lamina propria measured 66.6 by 50.3 μm (n = 50), while those in the liver were 70.9 by 55.0 μm (n = 5). Sexual stages were demonstrated in epithelial cells of the small intestines only. Macrogametocytes measured 9.1 - 14.4 by 5.0 - 14.2 μm (n = 15). Macrogametes measured 10.1 - 15.6 by 7.2 - 9.6 μm (n = 15) and microgametocytes measured 9.1 - 14.0 by 6.2 - 11.8 μm.(n = 10). Therefore the development of *Besnoitia wallacei* in the cat involves asexual stages in the intestines and the liver and sexual stages in the intestines.

Twenty cats were investigated for their involvement in the cyclic transmission of the *Besnoitia* species affecting goats in Kenya. Eight cats were fed heavily infected goat tissues, two cats were orally inoculated with 1 x 10^6 *Besnoitia* bradyzoites from the same goats and ten cats were under natural or artificial stress and fed on infected goat tissues or inoculated with 1 x 10^6 *Besnoitia* bradyzoites. None of the above cats produced oocysts in their faeces for 30 days post-infection. This indicated that the domestic cat plays no role in the transmission of the *Besnoitia* species affecting goats in Kenya.

Laboratory mice and rats were investigated for their role in transmission of the *Besnoitia* species affecting goats in Kenya. In ten mice and ten rats injected

intraperitoneally and ten mice and ten rats injected subcutaneously with bradyzoites from goat tissues, neither tissue cysts nor tachyzoites could be demonstrated. Six cats fed on their carcasses did not produce *Besnoitia* oocysts in their faeces for 30 days post-infection again indicating that mice and rats are not involved in the transmission of this *Besnoitia* species.

The infectivity of *Besnoitia wallacei* oocysts to goats was investigated. Five, 9-12 month-old goats orally inoculated with approximately 1 x 10^6 sporulated oocysts did not develop a clinical disease. Microscopically, mononuclear cell infiltration was observed in the lungs and liver, but cysts and tachyzoites could not be demonstrated. Two of the three cats fed on tissues from these goats produced oocysts of *Besnoitia wallacei* on day 12 of infection proving that the goat is a poor intermediate host for *Besnoitia wallacei*.

CHAPTER ONE

1. INTRODUCTION

1.1 GENERAL INTRODUCTION

The goat *(Capra hircus)* was among the first animals to be domesticated by man. They originated from Asia, but have since spread over all the continents. They inhabit all climatic zones from the arctic circle to the equator.

The goat population is largely concentrated in tropical and subtropical areas of Africa and Asia, and in the warmer temperate regions around the Mediterranean and Central America (Devendra and Mcleroy, 1982; Wilkinson and Stark, 1987).

The goat production industry is not well developed. In most countries, goat keeping has been associated with poverty and consequently they have been neglected in some of the countries development plans. However, since the late sixties, there has been a gradual change in this attitude and planners are becoming increasingly aware of the potential of the goat as a source of meat, milk, skin, fibre and as a laboratory animal. Governments and International agencies are providing new support for the education of goat owners, promoting scientific research on goats and improvement of their production schemes.

Goats are relatively resistant to diseases and are rarely seen suffering from minor disturbances. They become visibly sick only when seriously affected (Gall, 1981). This and the fact that goats have not received proper attention from owners and scientists have contributed to failure in diagnosis of certain diseases that affect them. One such disease is caprine besnoitiosis.

Besnoitiosis is a protozoal disease caused by the tissue cyst-forming coccidia of the genus *Besnoitia*. *Besnoitia* species have a wide range of natural and experimental intermediate hosts. The species affecting goats is not yet been classified, but is suspected to be *Besnoitia besnoiti*.

Besnoitiosis has been affecting goats in Kenya from as early as 1955 (Bwangamoi, 1968) as demonstrated in dried skin sections. However, the disease had not been clinically diagnosed the world over. It was not until 1979 that the first clinical case was diagnosed in wild goats (*capra aegagrus*) in Iran (Cheema and Toofanian, 1979).

The disease in goats was thought to be of no economic importance until 1989 when there was an out break in Marimanti and Kiburine sheep and goat projects in Kenya (Bwangamoi, Carles and Wandera, 1989). The disease was associated with infertility, neonatal deaths and abortions and caused skin damages. Since then it has been reported in Embu, Isiolo, Meru, Kitui, Nakuru and Turkana districts of Kenya (Bwangamoi, 1989).

To date, there is no effective treatment for besnoitiosis and the only effective control method is slaughter and disposal of all infected carcasses. The disease is therefore a great threat to the maintenance and expansion of the goat production industry and it's control is of utmost importance. An understanding of the life-cycle and the mode of transmission of the causative agent (*Besnoitia*) is important when designing and effecting a control programme.

The natural mode of transmission of *Besnoitia* species is not yet clear. It is thought to be transmitted mechanically by blood sucking arthropods and through carnivorism.

Bigalke (1968) successfully transmitted *Besnoitia besnoiti* from chronically infected cattle to rabbits and other susceptible cattle. He also demonstrated that *Glossina brevipalpis, Stomoxys calcitrans*, Tabanids, and mosquitoes can act as mechanical vectors. However, mechanical transmission alone can not explain the high incidence rate of the disease during outbreaks. The possibility of biological vectors has been suggested, but no developmental stages have been found in the insects (Bigalke, 1968).

Carnivorism is suspected to be the main mode of transmission of *Besnoitia* species. The domestic cat (*Felis catus*) is thought to be the main vector (final host).

The cat is involved in the cyclic transmission of *Besnoitia besnoiti* of cattle, impala, kudu and wildebeest (Peteshev, Galuzo and Polomoshnov, 1974), *Besnoitia darlingi* of lizards and opossum (Smith and Frenkel, 1977, 1984) and *Besnoitia wallacei* of house mouse, polynesian rats and Norway rats (Wallace and Frenkel, 1975; Fayer, 1980). The cat acquires infection by ingesting cysts in infected tissues of the intermediate hosts. The parasite multiplies in the small intestines leading to formation of oocysts. The oocysts are voided in faeces. The intermediate hosts become infected when they ingest sporulated oocysts in contaminated water or feeds.

It is therefore suspected that the cat is the final host for the *Besnoitia* species affecting goats in Kenya

1.2 OBJECTIVES

1. To identify the coccidia of cats around Kabete.
2. To study the development of *Besnoitia* in the cat.
3. To study and determine whether that the domestic cat is the final host for the *Besnoitia* species affecting goats in Kenya.
4. To study and determine whether *Besnoitia wallacei* oocysts from cat faeces can infect goats.

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CHAPTER TWO

2 LITERATURE REVIEW

The domestic cat (*Felis catus*) is the final host for five genera of coccidian parasites, *Besnoitia, Toxoplasma, Hammondia, Sarcocystis* and *Isospora*. It is involved in the transmission of 11 species, namely *Toxoplasma gondii* , *Hammondia hammondi, Besnoitia besnoiti. Besnoitia darlingi, Besnoitia wallacei, Isospora felis, Isospora rivolta, Sarcocystis hirsuta, Sarcocystis tenella, Sarcocystis porcifelis and Sarcocystis muris*

2.1 GENUS: *BESNOITIA*

2.1.1 CLASSIFICATION.

The classification of *Besnoitia* has been confusing and has undergone many revisions in the past. The confusion was mainly caused by inadequate information on its life-cycle as well as that of related gastrointestinal coccidia. *Besnoitia* was discovered by Besnoit and Robin (1912) from cattle and was named *Sarcocystis besnoiti* by Marotel (1912). Henry (1913) created the genus Besnoitia and named it *Besnoitia besnoiti* (Marotel, 1912). Brumpt (1913) named it *Gastrocystis besnoiti* (Marotel, 1912). However, Noller (1920) changed the name to *Globidium besnoiti* (Marotel, 1912).

Henry and Masson (1922) and Bennett (1927) named it *Globidium leukarti*. Babudieri (1932) and Reinchenow (1953) revised the classification of *Sarcosporidia* and *Globidium* respectively and renamed the parasite *Besnoitia besnoiti* (Marotel,

1912) and *Besnoitia bennetti* (Babudieri, 1932) for the cattle and horse species respectively.

Opinions differ on the family and subfamily assignment of the parasite. Frenkel (1974) placed it in the family *Toxoplasmatinae* (Biocca, 1956) on the basis of it's life-cycle, but Levine (1977) placed it in the family *Eimeridae*. Frenkel (1977) reclassified the cyst-forming isoporoid coccidia into the family *Sarcocystidae* and recognised two subfamilies, *Sarcocystinae* (Poche, 1913) and *Toxoplasmatinae* (Biocca, 1956).

The classification used here is that proposed by Frenkel (1977) and adopted by Soulsby (1982).

Phylum:	*Apicomplexa*, Levine, 1970
Class:	*Sporozoea*, Leuckart, 1879
Subclass:	*Coccidia*, Leuckart, 1879
Order:	*Eucoccidiidae*, Leger and Dubosq, 1910
Suborder:	*Eimeriina*, Leger, 1911
Family:	*Sarcocystidae*, Poche, 1913
Subfamily:	*Toxoplasmatinae*, Biocca, 1956
Genus:	*Besnoitia*, Henry, 1913
Species:	*Besnoitia besnoiti* (Marotel,1912) Henry, 1913
	Besnoitia bennetti (Babudieri, 1932)
	Besnoitia darlingi, (Brumpt, 1913) Smith and Frenkel, 1977
	Besnoitia jellisoni (Frenkel, 1955)

Besnoitia tarandi (Hadwen,1922) Levine, 1961

Besnoitia wallacei (Tadros and Laarman, 1976)

2.1.2 LIFE CYCLE AND TRANSMISSION

The natural mode of transmission of members of the genus *Besnoitia* has not been well elucidated. However, under natural and experimental conditions, three modes of transmissions have been observed, and include

1) The transmission of tissue cysts from intermediate hosts to the final hosts.
2) The transmission of oocysts from the final host to the intermediate host and
3) The transmission of the tachyzoites or bradyzoites from one intermediate host to another.

The transmission of tissue cysts from one intermediate host to the final host and that of the oocysts from the final host to the intermediate host has been successful in *Besnoitia besnoiti* (Peteshev, *et al.*, 1974), *Besnoitia darlingi* (Smith and Frenkel, 1977, 1984) and *Besnoitia wallacei* (Wallace and Frenkel, 1975; Ito, Tsunoda and Shimura, 1978; Fayer,1980). These species have an obligatory two-host cycle, with the cat as the final host and a wide range of natural and experimental intermediate hosts.

The cat gets infected when it ingests cysts in tissue of intermediate hosts. Bradyzoites are released from the cysts and schizogony initiated in the epithelial cells and lamina propria of the small intestines (Wallace and Frenkel, 1975; Frenkel, 1977; Ito *et al.*, 1978). Frenkel (1977) found schizont of *Besnoitia wallacei* in the lamina propria of the small intestines of cats between 4 and 14 days of infection. The

schizonts measured between 500 and 800 μm when mature. Similar schinzonts were reported by Ito *et al* (1978) between day 6 and 19 post-infection, but were smaller (50 - 120 by 35 - 80 μm). They generally extended into vessels and appeared to be in endothelial or intimal cells. Frenkel (1977) also observed schizonts in the liver after 10 days of infection. At least one generation of schizont has been observed (Fayer, 1980), but the number of generations of schizogony is not yet clear. Macrogametocytes measuring 10 to 13 μm were observed on day 13 to 16 of infection in Goblet cells of small intestines, usually between the vacuole and nucleus. They were larger than those of *Toxoplasma gondii* and *Hammondia hammondi* (Frenkel and Dubey, 1975) and *Sarcocystis muris* (Ruiz and Frenkel, 1976) but smaller than those of *Isospora felis* (Shah, 1971). Microgametocytes measuring upto 11 μm were identified on day 13 of infection.

Macrogametes and microgametes fuse to form oocysts which are latter extruded into the intestinal lumen. They are passed out in faeces after 12 to 15 days of infection. Patency lasts 5 to 12 days (Frenkel, 1977). The prepatent periods for *Besnoitia besnoiti* and *Besnoitia darlingi* are 13 to 16 days and 11 to 14 days respectively. Patency last for 5 to 8 days for *Besnoitia darlingi* (Smith and Frenkel 1977; McKenna and Charleston, 1980b). Cats infected with *Besnoitia wallacei* will produce oocysts in subsequent infections (Wallace and Frenkel, 1975). However this is not the case with *Besnoitia darlingi* (Smith and Frenkel, 1977).

The oocysts of *Besnoitia* species are shed unsporulated and sporulate outside the host to become disporic oocysts with tetrazoic sporocysts. The intermediate hosts become infected following ingestion of sporulated oocysts in contaminated feed or

water. This is followed by the development of cysts in their tissues. Oocysts of *Besnoitia* species are not infectious to the final host (Wallace and Frenkel, 1975).

The transmission of *Besnoitia* species through tachyzoites and bradyzoites from one intermediate host to another has been achieved under experimental conditions. The tachyzoites of *Besnoitia besnoiti* have been transmitted during the acute (febrile) stages of the disease to susceptible cattle and rabbits by intravenous and intraperitoneal injection of infective blood (Cuille, Chelle and Berlureau , 1936; Pols, 1954a) or by intradermal, subcutaneous and intravenous injection of pooled emulsions of the spleen and lymph nodes (Schulz, 1960). The bradyzoites recovered from chronically infected cattle and antelopes have been transmitted into susceptible cattle, rabbits, goats, gerbil and hamsters by intraperitoneal, intravenous and subcutaneous injection (Bigalke, 1967; Bigalke, Van Niekerk, Basson and McCully, 1967; Diesing, Heydorn, Matuschka, Bauer, Pipano, De Waal and Potgieter, 1988).

Haematophagic arthropods can act as mechanical vectors for *Besnoitia besnoiti* as demonstrated by Bigalke (1968) who successfully transmitted bradyzoites from chronically infected cattle to susceptible cattle and rabbits, through *Glossina brevipalpis, Stomoxys calcitrans*, Tabanid flies and mosquitoes. He also observed that cohabitation of chronically infected cattle with susceptible ones resulted in mild infection of the latter group.

Besnoitia darlingi and *Besnoitia jellisoni* have been transmitted by intraperitoneal injection of tachyzoites obtained from mice peritoneal washing (7 days following infection) into lizards, rats, mice, opossums and hamsters (Schneider, 1967a; Senaud, Mehlhorn and Scholtyseck, 1974) and by oral inoculation into marmosets (Schneider, 1967c). *Besnoitia tarandi* has been transmitted by intravenous

injection of bradyzoites from chronically infected caribou to susceptible caribou and mule deer (Glover, Swendrowski and Cawthorn,1990). However, bradyzoites of *Besnoitia wallacei* are not readily transmissible from one intermediate host to the other. They are only poorly transmissible to *Ratus exulans* (Frenkel, 1977).

The sequential development of *Besnoitia* species in the intermediate host is not yet clear. Following the introduction of bradyzoites or tachyzoites into the intermediate host, they are taken up by monocytes and macrophages where they multiply by endodyogeny (Frenkel, 1953; Pols, 1954a,b; Senaud *et al.*, 1974). These cells then rupture and release parasite which infect histiocytes and multiply by endodyogeny resulting in cyst formation (Pols, 1960; Senaud *et al.*, 1974; Basson, McCully and Bigalke, 1970; Gobel, Widauer, Reimann and Munz, 1985).

2.1.3 IDENTIFICATION.

Besnoitia is identified by its characteristic polyzoic cysts with thick periodic acid schiff (PAS) positive walls. (Chobotar, Anderson, Ernst and Hammond, 1970 ; Fayer,1980). The mature cyst walls are composed of three distinct parts.

a) The outer layer of dense, hyaline substance, the inner surface of which sometimes appear to stain darkly.

b) A layer of cyst wall cytoplasm and hyperplastic and hypertrophic nuclei of host cell origin and

c) A thin inner membrane which is in intimate contact with the bradyzoites (Schneider, 1965; Sheffield, 1968; Sagiev, 1981).

The cysts are usually found in connective tissue and are visible to unaided eye. These characteristics can be used to differentiate *Besnoitia* cysts from those of other tissues

cyst forming coccidia. Cysts of *Sarcocystis* are macroscopic or microscopic. They are chambered or entire. The cyst wall is thin and denticulate, PAS negative and host cell nuclei are absent. Cysts of *Toxoplasma* and *Hammondia* are microscopic, they have a thin denticulate membrane which is PAS positive, but contain no host cell nuclei (Garnham, 1966). The cysts of *Isospora* have a thick PAS positive wall but contain a single zoite (Frenkel and Dubey, 1972).

The morphology and size of *Besnoitia* cysts vary according to *Besnoitia species*, host species, location and stage of development. The cysts of *Besnoitia bennetti* in Mexican burros are spherical and measure 400 to 550 μm. The cyst wall is 10 to 15um thick (Terrell and Stookey, 1973). The cysts of *Besnoitia besnoiti* in cattle are spherical and measure 100 to 600 μm in diameter (Bwangamoi, 1979). *Besnoitia darlingi* cysts in the opossum measure 438 to 827 μm (Smith and Frenkel, 1977). The cysts of *Besnoitia jellisoni* in opossum are spherical and measure 685 to 754 by 514 to 548 μm (Stabler and Welch, 1961). Those of *Besnoitia tarandi* in the caribou are spherical and measure 240 to 373 μm in diameter. The cyst wall measures 14 to 56 μm (Glover *et al.*, 1990). The cysts of *Besnoitia wallacei* in mice are spherical and measure 90 to 210 μm in diameter. The cyst wall measures 1 to 30 μm thick (Wallace and Frenkel, 1975). Cysts of the *Besnoitia species* affecting goats in Kenya measure 150 to 350 μm in diameter (Bwangamoi, 1989).

Oocyst morphology can also be used for the identification and differentiation of *Besnoitia* species (Fayer, 1980). The unsporulated oocyst of *Besnoitia wallacei* are subspherical, and measure 16 to 19 μm by 10 to 13 μm. The oocyst wall is bilayered, about 0.5 μm thick. Micropyle and polar granules are absent. Sporonts are finely granular, light brown in colour and initially filling oocyst almost completely. When

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sporulated in 1 or 2% sulphuric acid, at 24 ± 1° C, agitated and exposed to air, sporulation is complete in 48 to 96 hours (Wallace and Frenkel, 1975; Frenkel, 1977). After sporulation, the inner wall layer frequently separates partially from the outer layer. Sporulated oocysts are elliptical to subspherical and measure 16 by 13 μm. Each sporulated oocyst contains two elliptical sporocysts, 11 by 8 μm and lack a stieda body. The sporocysts contain four sporozoites 10 by 2 μm each (Frenkel, 1977).

The unsporulated oocysts of *Besnoitia darlingi* measure 11.2 to 12.8 μm by 10.8 to 12.8 μm and contain a finely granular sporont which almost fill the oocyst cavity. They have no micropyle or polar granules. When sporulated they contain two elliptical sporocysts without oocyst residuum. Sporulated oocysts measure 11.2 to 12.8 by 9.6 to 11.2 μm. Sporocysts measure 6.2 to 8.9 μm by 4.8 to 6.2 μm. Each sporozoite measures approximately 5 by 2 μm. The sporulation time is 48 to 72 hours on exposure to air at room temperature under experimental conditions (Smith and Frenkel, 1977, 1984).

The unsporulated oocyst of *Besnoitia besnoiti* measure 15 by 13 μm, with a range of 14 to 16 by 12 to 14 μm (McKenna and Charleston, 1980b).

Serological tests and host specificity can also be used for identification and differentiation of *Besnoitia* species.

2.1.4 EPIDEMIOLOGY

Besnoitiosis was first described from a pyrenean cow by Besnoit and Robin (1912) although the disease was known to occur in the 19th century (Bwangamoi, 1979). *Besnoitia* species have since been reported in many parts of the world.

Besnoitia besnoiti has been reported in South-Western Europe Asia and Africa where it affects cattle and wild ungulates (Basson, Van Niekerk, McCully and Bigalke, 1965; Bwangamoi, 1969,1979; Neuman, 1972).

Besnoitia bennetti of horses, burros and donkeys has been reported in Europe, America and Africa (Henry and Masson, 1922; Schulz and Thorburn, 1955; Bwangamoi, 1972; Terrell and Stookey, 1973). *Besnoitia jellisoni* of kangaroo rats, mouse and opossum has been reported in North America (Frenkel, 1955). *Besnoitia darlingi* of lizards and opossum has been reported in America (Garnham, 1966; Schneider, 1965, 1967b) *Besnoitia tarandi* of the Alaskan reindeer, Swedish reindeer and caribou in America and Sweden (Hadwen, 1922; Choquette, Broughton, Miller, Gibbs and Cousineau, 1967; Rehbinder, Elvander and Nordkvist, 1981). *Besnoitia wallacei* of mice and rats has been reported in America, Australia, New zealand and Japan (Wallace and Frenkel, 1975; Ito *et al.*, 1978; McKenna and Charleston, 1980b; Mason, 1980).

Other unclassified species have been reported in various parts of the world. This includes *Besnoitia* oocyst recovered from cat faeces in Mexico (Pacheco, 1986), camel *Besnoitia* in India (Kharole, Gupta and Singh, 1981), Opossum *Besnoitia* in Brazil (Naiff and Aris 1983), *Besnoitia* species in goats in Iran and Kenya (Bwangamoi, 1967, 1968; Kaliner, 1973; Cheema and Toofanian, 1979; Bwangamoi *et al* 1989). *Besnoitia* of the Blue duicker has been reported in USA (Foley,Anderson and Steinberg, 1990). Suspect *Besnoitia* species have been reported in flamingos in Kenya (Karstad, Sileo and Hartley, 1981) and in Knots in USA (Simpson, Woodlard and Forrester, 1977).

Besnoitiosis in Kenya was first diagnosed in goat skin by Bwangamoi(1967). However, the disease was in existence as early as 1955 as evident from dried skin sections (Bwangamoi, 1968). In 1973, Kaliner observed *Besnoitia* cysts in lung section in Kenya. It was not until 1979 that the first clinical case was reported in wild goats (*Capra aegagrus*) in Iran (Cheema and Toofanian, 1979). Heydorn; Senaud, Mehlhorn and Heinonen, (1984) observed *Besnoitia* cysts in the eyelids of goats in Kenya.

The disease in goats was thought to be of no economic importance until 1989 when there was an outbreak in Marimati and Kiburine sheep and goat projects in Kenya (Bwangamoi *et al.*, 1989). The disease was associated with infertility, neonatal deaths, abortion and skin damages. Since then the disease has been reported in Embu, Isiolo, Meru, Kitui, Nakuru and Turkana districts of Kenya (Bwangamoi, 1989).

Detailed information of the epidemiology of caprine besnoitiosis is lacking. More information is available for bovine and rodent besnoitiosis. Bovine besnoitiosis is most common in low lying areas, especially where surface water is abundant (Schulz, 1960). It has a seasonal occurrence with most outbreaks occurring during warm wet seasons. At this time, the bitting flies which are thought to be mechanical vectors are in plenty (Bigalke, 1968). Susceptibility of cattle increases with age. There is no breed specificity in cattle, but management factors may lead to higher incidence in beef breeds than in dairy breeds (Goldman and Pipano, 1983). In goats, Bwangamoi *et al.*, (1989), reported a higher incidence of ocular cysts in Gala goats than in Toggenburgs. The morbidity rate in large herds of cattle is up to 50 percent, and may be as high as 100% in smaller herds (Schulz, 1960). The mortality rate is between 5 and 20%.

Besnoitia as a parasite has evolved different mechanisms of survival both in and outside the host. The development of diapause stages by *Besnoitia wallacei*, *Besnoitia darlingi* and *Besnoitia besnoiti* (oocysts in the cat and tissue cysts in the intermediate hosts) ensure their immediate survival and enable their disposal and thereby their continuity.

Oocysts produced by the cat are only infectious after sporulation. Sporulation is influenced by environmental conditions especially temperature, moisture and aeration (Fayer, 1980). Temperature extremes, low humidity and low oxygen tension retard sporulation rate. Both sporulated and unsporulated oocysts can remain viable for a long time. They are extremely resistant to effects of harsh chemicals and are routinely cleaned of faecal debris and disinfected from bacterial contaminations before use *in vitro* studies by suspension in 2% sulphuric acid or 2.5% potassium dichromate. *Besnoitia wallacei* oocyst can survive in 2% sulphuric acid at 4°C for up to 38 months (Frenkel, 1977).

Due to their relatively small size and resistance to the environmental factors except heat and drying, oocysts may easily be dispersed by wind, water or transported on clothes, boots or hands of animal handlers. They can also be transported by other animals from one place to another. *Toxoplasma* has been transmitted experimentally by earthworms and filthy flies (Dubey, Miller and Frenkel, 1970; Frenkel, Ruiz and Chinchilla, 1975; Wallace, 1971).

In the intermediate host, the parasite invades macrophages and also forms thick-walled cysts, thereby evading the host defensive system (Senaud *et al.*,1974; Muel, 1984). The tissue cysts in the intermediate hosts remain viable for long

periods of time, during which they are potentially infective for the final and other intermediate hosts (Pols, 1960).

The tissue cysts are also resistant to harsh chemicals and environmental conditions other than direct sunlight and heat (Uvaliev and Baigaziev, 1976; Polomoshnov, Umbetaliev and Peteshev, 1979). In dead animals, they can remain viable until the carcasses decompose and when frozen they remain viable for upto 8 months (Polomoshnov *et al.*, 1979). Infected tissues and contaminated environment can be sources of infection.

Wildlife are extremely important in the epidemiology of coccidial infections. Some wildlife species are closely related to domestic animals and share species or strains of parasite. This is the case with *Besnoitia besnoiti* which affects cattle, kudu, impala and wildebeest. The disease problems usually arise when the territories of the wild animals and domestic animals overlap, or when vectors or transport hosts have access to both groups (Fayer,1980).

2.1.5 HOST SPECIFICITY

Besnoitia species are capable of parasitizing a wide range of natural and experimental intermediate hosts. The final host is the cat and the intermediate hosts range from the cold blooded reptiles to the worm blooded mammals.

Besnoitia bennetti, has the natural intermediate hosts as the horse, donkey and burros. The final host is unknown.

Besnoitia besnoiti, has the natural intermediate hosts as cattle, impala, kudu, and wildebeest. The experimental intermediate hosts include goats, gerbils, rabbits, sheep and hamsters (Pols 1960; Bigalke, 1968). Discrepancies exist in the literature

concerning the susceptibility of mice, guinea pigs and rats. Neuman (1965) and Peteshev *et al* (1974) reported susceptibility of mice with the Asian strain of *Besnoitia besnoiti*, but Pols (1960), Kaggwa, Weiland and Rommel (1979) and Shkap, Pipano and Greenblatt (1987a) reported resistance of mice, rats and guinea pigs. Also Pak (1976) could only recover tachyzoites from peritoneal washing and visceral organs of mice upto day 18 of infection, but could not demonstrate cysts in their tissues 3 months post-infection.

The definitive host is the cat (Peteshev *et al.*, (1974). However, attempts by Rommel (1975) to transmit the parasite orally from infected cattle to dogs, cats, a serval, vultures, and marabous in Uganda were inconclusive. Similarly, Diesing *et al* (1988) attempted to transmit the parasite orally to domestic cats, jungle cats, caracal, small spotted genet, marsh mongoose, domestic dog, cat snake, montpelier snakes, palestinian viper, European whip snake, Ravergiers whip snake, puff adder, lion, leopard, cheetah, Banded mongoose, black-backed jackals, cape fox, and white backed vulture without success.

Besnoitia darlingi whose natural intermediate hosts are lizards and opossums can experimentally infect hamsters, marmosets, squirrels, mice and bats (Schneider, 1967,b,c, Fayer,1980, Smith and Frenkel, 1984). The final host is the cat (Smith and Frenkel, 1977,1984).

Besnoitia jellisoni has the natural intermediate host as the kangaroo rats, mice, and opossum. The experimental intermediate host include guinea pig, hamsters, mice, and rats (Sheffield 1968; Conti-Diaz, Turner, Tweeddale and Furcolow, 1970; Senaud *et al.*, 1974). The final host is unknown. Attempts by Wallace and Frenkel(1975) to

transmit the parasite to cats, bobcats, cougar, foxes, dogs, coyote, procyonids, skunk, owl, hawk, bard, colubrid, viperid snakes were unsuccessful.

Besnoitia tarandi has the natural intermediate host as the caribou and reindeer. An attempt by Glover *et al.*, (1990) to transmit the parasite to Chinese water deer, mule deer, white tailed deer, forsmosan sika deer, sheep, and cattle were unsuccessful. The final host has not yet been identified. Attempts to transmit the parasite to domestic cats, raccoon and arctic fox were unsuccessful (Glover *et al.*, 1990).

Besnoitia wallacei has the natural intermediate host as the house mouse and rat while experimentally the intermediate hosts are gerbils, vole and rabbits (Wallace and Frenkel, 1975; McKenna and Charleston, 1980b). Infection of guinea pigs was unsuccessful (Wallace and Frenkel, 1975;Ito *et al.*, 1978; McKenna and Charleston, 1980b). Hamsters are poorly susceptible to *Besnoitia wallacei* infection. Although Ito *et al* (1978) could not demonstrate tissue cysts, they recovered oocysts from cats fed on the hamster's tissues.

2.1.6 CLINICAL SIGNS.

The clinical manifestation of caprine besnoitiosis has not been well studied. However, the cases reported by Cheema and Toofanian (1979) and Bwangamoi *et al* (1989) were advanced and resembled chronic besnoitiosis in cattle and other domestic and wild animals.

The experimental incubation period of bovine besnoitiosis is 6 to 10 days (Pols, 1960). There are three stages in the development of the disease, namely, the acute stage (febrile, primary), the subacute stage (depilatory, secondary) and the

chronic stage (seborrhoea sicca, tertiary), (Hofmeyer, 1945; Pols, 1960; Schulz, 1960).

During the acute stage, there is high fever of upto 41.6°C. This is accompanied by photophobia, lacrimation, hyperaemia of the sclera, staring coat, anorexia, depression and listlessness. There is painful subcutaneous oedematous swelling affecting any part of the body, but with predilection for one or more limbs, scrotum, dewlap and flank. Oedema appears after the peak fever (Pols, 1960; Diesing *et al.*, 1988). There is enlargement of lymph nodes, stiff gait and the animal is lethargic. Pregnant animals may abort. In severe cases, there is haemorrhagic mucopurulent rhinitis, swelling of the nasal mucosa and encrustation of exudate in the nasal passages and nares leading to difficult breathing. Coughs may be observed in cases of pharyngeal and laryngeal involvement (Schulz, 1960; Glover *et al.*, 1990).

The subacute stage is the critical phase on which the prognosis is based. There is pronounced scleroderma with excessive thickening of the skin and loss of elasticity. Subsequently, there is excessive skin folding, cracking alopecia and oozing of sero-sanguineous exudate (Pols, 1960: Diesing *et al*, 1988). This leads to secondary bacterial infection which is often responsible for deaths. The subacute stage lasts 2 to 4 weeks and may end in death due to debility and exhaustion.

Animals surviving the depilatory stage progress to the chronic stage in which there is loss of hair from the formally anasarcous areas and formation of thick scurfy scales which fall off as fine flakes. The lymph nodes remain permanently enlarged. The severely affected cases remain emaciated, with thickened and wrinkled skin for life. Recovered animals remain carriers for life. Infected bulls become permanently sterile. The testicles are atrophic and firm (Pols, 1954a, 1960).

Rodents infected by *Besnoitia darlingi* show a characteristic fall in temperature, rough hair coat and occasional diarrhoea which becomes evident a day before death (Schneider, 1967a).

2.1.7 PATHOLOGY

The pathology of caprine besnoitiosis has not been well studied. However, the cases reported by Cheema and Toofanian (1979) and Bwangamoi *et al* (1989) resembled chronic besnoitiosis in cattle, other domestic and wild animals.

2.1.7.1 GROSS PATHOLOGY

The gross pathological changes vary according to the duration, intensity and extends of the infection in individual cases. In bovine, lesions of the skin, mucous membranes of the upper respiratory system, cornea, the subcutaneous and interstitial connective tissues of the skeletal muscles and testes dominate (Schulz, 1960).

In acute cases the tegumentary system is characterised by a dull coat, generalized anasarca, which is more pronounced in lips, muzzle, face, ears, neck, brisket, abdomen, flanks, and limbs (Schulz, 1960; Basson *et al.*, 1970). There is subcutaneous congestion, haemorrhages, and thrombosis as well as erosions on the lips and muzzle.

Lymph nodes and haemonodes are usually enlarged, oedematous, congested and with a few foci of haemorrhage and necrosis. The mucosa of the upper respiratory system may be covered by pseudomembranes and may be congested, oedematous, necrotic and may have a few haemorrhagic foci. In the lungs, severe alveolar and interstitial oedema can be seen (Basson *et al.*, 1970; Diesing *et al.*,

1988). The liver and kidney may be congested and show signs of degeneration. The skeletal muscles may have foci of degeneration and necrosis, while veins of joint capsules, tendons and hypophyses may be thrombosed. Occasionally fibrinous peritonitis and pleuritis are encountered. The testes in males are usually swollen and turgid (Pols, 1960).

In subacute cases, there is thickening, depilation, cracking, scurviness and crust formation of the skin as the most salient features (Schulz, 1960). The cracks and fissures mostly affect flexure surfaces. Decubitus wounds are often seen in recumbent animals.

In chronic cases, the skin is usually thickened, wrinkled, hyperkeratotic and has areas of alopecia. The subcutis on incision reveals numerous fine, white sand-like cysts which are approximately 0.25 to 0.5 mm in diameter (McCully, Basson, Van Niekerk and Bigalke,1966). There is serous atrophy of sub-cutaneous fat which indicates emaciation. The mucosa of the upper respiratory system is studded with numerous cysts and few cysts may be seen in the lungs (Bennett, 1933; Schulz and Thorburn, 1955; McCully *et al.*, 1966).

The veins especially those of the limbs and face are studded with cysts, giving the intima a granular appearance. The skeletal muscle, especially those of lower limbs have a whitish, mottled appearance and have numerous cysts. Cysts are also numerous in the joint capsules, tendons and periosteum of bones of lower limbs. In the eye, cysts are commonly seen in the bulbar conjunctiva. The lymph nodes are usually enlarged. In males the testes are usually firm and shrunken (Pols, 1960).

In goats, the skin may be rough, thick, alopecic, ulcerated and covered with scabs. White sand-like cysts may be seen in the subcutis of the neck, limbs, thoracic

region, tunica albuginea, epididymis, teats, intercostal muscles, fasciae, eyelids and bulbar conjunctivae, endothelium of venules and arterioles. Few cysts may also be seen in the lungs. (Cheema and Toofanian, 1979; Bwangamoi *et al.*, 1989).

In rodents infected with *Besnoitia wallacei*, cysts become grossly visible 4 to 6 weeks post-infection (Wallace and Frenkel, 1975). The cysts are mainly concentrated on the walls of the ileum, caecum, mesentery, omentum and peritoneum. A few cysts can be seen in the skeletal muscles, heart, brain, lymph nodes, thymus, pancreas, tongue, oesophagus, jejunum and anterior colon (Wallace and Frenkel, 1975; Frenkel 1977; Ito *et al.*,1978).

2.1.7.2 HISTOPATHOLOGY

During the acute stages of bovine besnoitiosis, cutaneous lesions are the most characteristic and striking. The epithelium is hyperplastic as shown by an increase of cells of stratum spinosum. There is pronounced hyperaemia and oedema, especially of the corium, perivascular cell infiltrations especially lymphocytes, plasma cells, fibroblasts and several large mononuclear cells (Schulz, 1960; Basson *et al.*, 1970). There are small haemorrhagic foci, necrosis of the intima and media and thrombosis of blood vessels supplying the skin. This leads to infarction and necrosis of the skin (Basson *et al.*, 1970). Free crescentic forms of *Besnoitia* can be seen in arterioles, lymphatics and in the spaces between the collagenous connective tissue fibres. Intracellular parasites can be seen at various developmental stages of the early cyst. (Schulz, 1960). The liver and kidney may show degenerative changes or few areas of necrosis. In the lung, signs of mild pneumonia, congestion, oedema and

emphysema may be seen. The lymph nodes are usually hyperplastic, congested, haemorrhagic and have few foci of necrosis.

In the subacute stages, the inflammatory changes become more pronounced. There is an increase in the population of mononuclear cells and a few neutrophils around the sites of parasite development. The cyst size increases markedly, and the host cell cytoplasm and nucleus are pushed peripherally. The skin shows variable degrees of hyperkeratosis, crust formation and wrinkling. There is hyperplasia of the epithelium, an increase in fibroblast population that leads to partial replacement of the collagenous connective fibres (Basson *et al.*, 1970). There is degeneration or necrosis and loss of hair. In the testes, there is extensive coagulative necrosis of seminiferous tubules, with dystrophic calcification and aspermia (Basson *et al.*, 1970).

In chronic cases, *Besnoitia* persists mainly as the mature or dormant cyst forms (Schulz, 1960). The number of cysts varies considerably depending on the degree of the infection. In milder cases, a few cysts, hair follicles and sheaths, some glandular tissues and cellular exudate are present. In severe cases of bovine and equine besnoitiosis, the corium is completely replaced by enlarged cysts, which are only separated by their hyalin capsules (Bennett, 1933; Schulz, 1960). There is hyperkeratosis, scab formation or secondary bacterial and fungal infection. There are granulomatous reactions, especially around ruptured cysts. In the cardiovascular system, cysts are seen in the intima, media adventitia or beneath the endothelium. Those beneath the endothelium often cause bulging of epithelium into the vessel lumen. Some cysts extend into the lumina with sessile or pediculate attachments. Some ruptured cysts are also seen. The freed organisms are clumped together in groups, especially in valves (McCully *et al.*, 1966).

In the respiratory system, the mucosa of the nasal passages, turbinate, larynx, and trachea may have cysts which invoke a mild host response. Few degenerate cysts can be seen in the lungs (Bennett, 1933; McCully *et al.*, 1966)

In the musculoskeletal system, many cysts can be seen in muscles, tendons and fibrous connective tissues, especially those of limbs. There is a severe host response in skeletal muscles. The periosteum of metatarsal and metarcapal bones may be heavily parasitised and a few cysts can be entrapped in the newly formed periosteal bone (McCully *et al.*, 1966). Such cyst entrapment has also been observed in caribou infected with *Besnoitia tarandi* (Glover *et al.*, 1990) and in skulls of rodents infected with *Besnoitia jellisoni* (Ernst, Chobotar, Oaks and Hammond, 1968; Wobeser,1976).

In the testes, cysts can be seen in the tunica vaginalis and tunica albuginea, in the walls and lamina of veins and arteries of the pampiniform plexus. The presence of cysts in this sites leads to thrombosis. Cysts can also be seen in interstitium and lamina propria of the epididymis, between and inside seminiferous tubules. Sertoli cells may be reduced in numbers and these may lead to aspermia. The host response may vary from none to granulomatous reactions (Pols, 1960; Terrell and Stookey, 1973; Cheema and Toofanian; 1979, Bwangamoi *et al.*, 1989; Foley *et al.*, 1990).

In mice infected with *Besnoitia wallacei* cysts often degenerate by day 70 of infection, leaving granulomas which can be 4 to 5 times the original size of the cysts. In rats the cysts can persist for over a year without degeneration (Frenkel, 1977.)

2.1.8 DIAGNOSIS

A tentative diagnosis of besnoitiosis can be made by considering certain epidemiological factors, the clinical signs and the lesions at autopsy. Confirmatory

diagnosis depends on the demonstration of *Besnoitia* microscopically. This can be achieved by careful examination of blood or tissue smears or in histological sections. Recently, serological tests which are sensitive and relatively specific for *Besnoitia* have been developed. In the final host, oocyst morphology and size can be used for tentative diagnosis. For confirmatory diagnosis animal inoculation is done.

The most important epidemiological factors to be considered are the geographical distribution of *Besnoitia* species, the seasonal occurrence of the disease and the natural intermediate and final hosts of the parasite (Pols, 1960; McKenna and Charleston, 1980b). These factors have been dealt with in details under the sub-topics epidemiology and host specificity.

The clinical signs commonly observed in besnoitiosis are fever of upto 41.6°C, anasarca, scleroderma, alopecia, seborrhoea and lymph node enlargement (Pols, 1960: Terrel and Stookey, 1973; Glover *et al.*, 1990). Careful inspection of the sclera for cysts can help detect clinical and subclinically chronic infections (Bigalke and Naude, 1962). At autopsy, the subcutis and other infected tissues reveal numerous fine, white sand-like cysts (McCully *et al.*, 1966).

During the acute stages of bovine besnoitiosis, microscopic examination of blood and lymph node smears may reveal the infectious agents. When the agents cannot be demonstrated from the smears, a biological test on rabbits should be undertaken (Pols, 1960). In chronic infections, the bradyzoites can be demonstrated in deep skin and scleral conjunctival scrapings and skin biopsy smears. The bradyzoites are banana shaped and measure 7.7 μm by 1.5 μm or as the stumpy form which measure 6.2μm by 3.1μm in size (Pols,1960; Sannusi,1991).

Pols (1960) states that a careful examination of histological sections from skin biopsies or other infected tissues usually reveals *Besnoitia* cysts at different stages of development. Mature cysts have a characteristic thick periodic acid schiff (PAS) positive wall. The host cell cytoplasm and nuclei are pushed peripherally and the nuclei are usually hyperplastic and hypertrophic.

A number of serological tests have been tried to aid in diagnosis of either experimental and natural besnoitiosis in various animals. The first serological test for bovine besnoitiosis was performed by Frenkel, (1953). He applied the Sabin-Feldman dye-test (SFT), using *Besnoitia jellisoni* as antigens, but failed to obtain positive reactions. However, Lunde and Jacobs (1965) demonstrated that the Sabin-Feldman dye test was highly specific for *Besnoitia jellisoni* while the haemagglutination test had cross reactions with *Toxoplasma gondii* antigens. Krasov and Omarov (1975) used *Besnoitia besnoiti* as antigens for haemagglutination, immunodiffusion and complement fixation tests and obtained positive results with sera from cattle, but observed cross-reaction with *Toxoplasma gondii* antigens. Kaggwa *et al* (1979) used indirect immunofluorescent antibody test (IFAT) and enzyme-linked immonosorbent assay (ELISA) in diagnosis of *Besnoitia besnoiti* and *B.jellisoni* infections in rabbits and mice. Janitschke, DeVos and Bigalke (1984) used the enzyme linked immunosorbent assay (ELISA) and immunofluorescence test in diagnosis of bovine besnoitiosis, they did not observe any cross reaction with *Anaplasma*, *Babesia*, *Theileria* or *Sarcocystis*.

Due to the high sensitivity and specificity of some of the above serological tests and the fact they can detect early and subclinical infections, they have been used for epidemiological surveys of bovine besnoitiosis. Frank, Klinger and Pipano,

(1970); Neuman (1972) and Goldman and Pipano, (1983) used immunofluorescence tests and recorded a high prevalence of *Besnoitia* infection in cattle in Israel. Basanets, Khvan, and Sokolovskaya (1978) used the complement fixation test (CFT) to detect infected cattle and observed 42 to 77 percent reactors, where only 10.7 percent were detected by scleral conjunctival cysts. Using the same test, Sagiev and Kamazanov, (1984) detected 33 out of 207 positive heifers where only 11 had been detected by clinical examination.

2.1.9 TREATMENT

No satisfactory chemotherapeutic agent has been found for the treatment of besnoitiosis in both the final and intermediate hosts. Leitao (1949) used three injections of 30ml of 10 percent formalin at 3 days interval in the treatment of chronically infected cattle. Herin (1952) advocated a single intravenous injection of 30ml of 1% formalin during the acute (febrile) stages and intravenous injection of 20 to 30mls of lugols iodine at 4 to 7 days interval for chronically infected cattle. Both reported beneficial effects. Claims by South African farmers that beneficial results were obtained by application of Benzene hexachloride preparations and or use of motor oil in treatment of cattle could not be substantiated (Pols, 1960).

Variable success has been achieved in the treatment of the bovine and antelope strains of *Besnoitia besnoiti* in different experimental animals. Pols (1960) found that rabbits responded well to 200 to 400mg of sulfamerazine if treated during the chronic stage of the disease. Goats responded to 1% Antimony but not to sulphanilamide complex (Lee, Lee and Moon, 1979). A single dose of long acting oxytetracycline at a dosage rate of 30mg/kg body weight was protective for rabbits (Shkap, De Waal

and Potgieter, 1985), and 200mg/kg body weight given intramuscular was protective to gerbils during the acute stages of the disease (Shkap, Pipano and Ungar-Waron, 1987b). Sulfadiazine and pyrimethamine are effective against murine besnoitiosis (Dubey, 1977).

No trials have been conducted in the treatment of besnoitiosis in the final host, but treatment of related coccidia in cats and dogs have been inconclusive. The activity of various sulphonamide against Isospora is low (Soulsby, 1982). Brumpt (1943) found that mepacrine at a dosage rate of 0.01g/kg body weight was effective for cats. However Dubey (1976) concluded that there are no effective drugs for the treatment of coccidiosis in dogs and cats.

2.1.10 PROPHYLAXIS

Effective prophylactic measures for the prevention of the spread of besnoitiosis are dependant upon the determination of the natural mode of transmission and the host range of *Besnoitia* species in nature. Prophylactic measures should be aimed at an early detection of the disease, reduction of the predator intermediate host contact, reduction in the contamination of the intermediate host environment, vector control, prevention of animal movement from infected to clean areas (quarantine), destruction of reservoirs and immunization of susceptible animals.

Various serological tests have been developed and used in the detection of besnoitiosis. Such tests include ELISA, CFT, IFAT and the Sabin feldman dye test, these are discussed under diagnosis.

Predators which may act as the final hosts of *Besnoitia* species can be kept off animals enclosures by effective fencing and by use of live traps. This is a very

expensive method and difficult to implement in large farms, but has been used in zoos (Glover *et al.*, 1990).

Infected carcasses should be destroyed or buried deeply and the environment disinfected. This is because *Besnoitia* cysts in infected animal carcasses and contaminated environments remain viable for a long period of time and can act as sources of infection (Uvaliev and Baigaziev, 1976).

The use of suitable arthropodicides to kill arthropod vectors and use of fly controlled barns during fly seasons may help reduce transmission of the disease by arthropod vectors (Pols, 1960; Glover *et al.*, 1990).

On diagnosing besnoitiosis in a farm, quarantine should be imposed. Infected animals should be isolated and kept in enclosures at least 100 meters away from the clean stock, to prevent spread by blood sucking arthropods (Bigalke, 1968). Sagiev and Kamazanov (1984) suggested that all infected animals be slaughtered. This can be very expensive especially in enzootic areas. Young animals should be separated from the older animals and from the infected stock (Glover *et al.*, 1990).

Vaccination of cattle has been tried with *Besnoitia besnoiti* originally isolated from wildebeest and grown on tissue cultures. Durable immunity to the clinical form of the disease was produced in 100% of the vaccinated cattle, but subclinical infection did occur (Bigalke, Basson, McCully, Bosman and Shoeman, 1974).

The other Coccidia of the cat are also reviewed with more emphasis on the life-cycle.

2.2 GENUS *TOXOPLASMA*

Toxoplasma gondii is an intestinal coccidium of felids with an unusual wide range of intermediate hosts. Infection by this parasite is common in many warm-blooded animals including man and monkeys (Dubey, 1976, 1977; Olubayo and Mwongela, 1978).

Although as many as 64% of cats in the United States and elsewhere have antibodies to *T.gondii*, only 1% or less shed oocysts at any give time (Dubey, 1976, McKenna and Charleston, 1980a). Human infections are equally high, and serological tests indicated 44% infection rate in USA (Behymer, Harlow, Behymer and Franti, 1973) while 55.7% have been reported in Kenya (Bakal, Khan and Geobloed, 1968).

2.2.1 LIFE-CYCLE

The cat gets infected by ingesting cysts in tissues of intermediate hosts or by ingestion of sporulated oocysts (Fayer, 1980). Bradyzoites are released in the small intestines. The bradyzoites penetrate the epithelial cells of the small intestines and initiate the formation of numerous generations of *Toxoplasma*. Five morphologically distinct types of *Toxoplasma* develop in intestinal epithelial cells before gametogeny begins (Dubey and Frenkel, 1972). The stages are designated type A to type E instead of generations because there are several generations within one *Toxoplasma* type. After entry into epithelial cells of the upper part of the small intestines, the bradyzoites lose their PAS granules and divide into two or three to form type A organism. This is the smallest of all the five asexual intestinal *Toxoplasma* types. The individual organisms measure 2 by 1 - 2 μm and the groups measure 4 - 5 by

2 - 3 μm. The type A organisms occur in groups of 2 to 3 in the jejunum 12 to 18 hours after infection.

Type B organisms are characterized by centrally located nuclei and prominent nucleolus. The cytoplasm appears dark blue in Giemsa stain and the organisms are bipolar. They appear in the jejunum and ileum 24 to 54 hours post infection. They appear in groups of 2 to 30. Single parasites measure 1 - 2 by 2 - 3 μm and groups measure 3 - 17 by 2 - 11 μm. Multiplication is assumed to be by simple endodyogeny and endopolygeny (Dubey and Frenkel, 1972).

Type C organisms are elongated, have subterminal nuclei and a strongly PAS positive cytoplasm. They occur in the jejunum and ileum 24 to 54 hours post infection and divide by schizogony. They occur in groups of 16 to 40. Individual organisms measure 2 - 4 by 1 μm and groups measure 9 - 17 by 9 - 11 μm (Dubey and Frenkel,1972).

Type D organisms are smaller than type C and contain only a few PAS positive granules. They appear to divide by endodyogeny, schizogony and by "splitting" of the merozoite from the main nucleated mass without leaving a residual body. They occur in groups of 2 to 35 organisms in the jejunum, ileum and colon 32 hours post infection until oocyst are shed. Single organisms measure 3 - 5 by 1 μm and groups measure 4 - 8 by 3 - 8 μm (Dubey and Frenkel, 1972).

Type E organisms resemble the subtype of the type D which divide by schizogony, but a residual body remain following division. They occur in the jejunum, ileum and colon 3 to 15 days post infection. Single parasites measure 3 - 5 by 1 μm and groups measure 4 - 5 by 3 - 8 μm.

The gametocytes occur throughout the small intestines, but most commonly in the ileum, 3 - 15 days post infection. They are found distal to the host epithelial cell nucleus, near the tips of villi of small intestines. The female gametes are subspherical and each contain a single centrally located nucleus and several PAS positive granules. Mature male gametocytes are ovoid to ellipsoidal in shape. When microgametogenesis takes place, the nuclei of the male gametocytes divide to produce 10 to 20 nuclei (Dubey and Frenkel, 1972). The microgametes are flagellate and motile and penetrate mature macrogametes resulting in fertilization and oocysts formation.

Unsporulated oocysts are subspherical to spherical and are 12 by 10 μm in diameter. The oocyst wall contains two colourless layers. Micropyle and polar granules are absent. The sporont almost fills the oocyst, and sporulation occurs outside the cat within 1 to 5 days depending upon aeration and temperature.

Sporulated oocysts are subspherical to ellipsoidal and are 13 by 11 μm in diameter. Each sporulated oocyst contains two ellipsoidal sporocysts without stieda body. Sporocysts measure 8 by 6 μm. A sporocyst residium is present, but lacks oocyst residium. Each sporocyst contains four sporozoites (Dubey, 1976). The intermediate hosts become infected by ingesting sporulated oocysts, resulting in tissue cyst formation.

2.3 GENUS *HAMMONDIA*

Hammondia hammondi is an intestinal coccidium of cats. The natural intermediate host is the goat (Shimura and Ito, 1986, 1987). However, Shimura and Ito (1987) did not observe lesions, tachyzoites or cysts in goats killed after 15 days

of infection (with oocysts), but cats fed pooled tissues from these goats produced oocysts. Similarly, dogs infected with oocysts did not have lesions nor *Hammondia* developmental stages, but cats which fed on their tissues produced oocysts (Dubey, 1975).

The experimental intermediate hosts include; white mice, deer mice, multimamate rats, guinea pigs, rats, hamsters, and dogs (Dubey, 1976).

Structurally *H. hammondi* resembles *Toxoplasma gondii*. However, unlike *Toxoplasma* it has an obligatory two host cycle.

2.3.1 LIFE-CYCLE

Hammondia has an obligatory two host life cycle. Cats become infected by ingesting cysts in tissues of infected intermediate hosts. Bradyzoites are released in small intestines and schizogony initiated in the epithelial cells. The schizonts occur at 96 hours post infection and resemble those of *Toxoplasma* types D and E (Frenkel and Dubey, 1975). The subsequent development is as for *Toxoplasma*.

Unsporulated oocysts measure 13 by 11 μm. They sporulate in two to three days at 22°C-26°C. The oocysts are impossible to distinguish structurally from those of *Toxoplasma gondii*. Intermediate hosts become infected by ingesting sporulated oocysts, but cats cannot become infected through ingesting sporulated oocysts.

2.4 GENUS *SARCOCYSTIS*

Sarcocystis has an obligatory two host cycle, a carnivore is the final host and forms cysts in the muscles of the intermediate hosts which include reptiles, birds, small rodents and herbivores (Dubey, 1977). Different species have different final

hosts. The cat is the final host for 5 types of *Sarcocystis*. *Sarcocystis hirsuta* from cattle, *S. tenella* from sheep, *S. porcifelis* from pigs, *S.muris* from mice and a *Sarcocystis* species of Grant's Gazelle (Dubey, 1976).

2.4.1 LIFE-CYCLE

Cats become infected by ingesting mature cysts in the tissues of intermediate hosts. The bradyzoites are released and penetrate the lamina propria of the small intestines to form gametes without producing schizont (Ruiz and Frenkel, 1976). Microgametocytes of *S.muris* measure 6.5 μm (Ruiz and Frenkel, 1976). The male gametes fertilize female gametes to produce oocysts. The oocysts sporulate in the lamina propria, two sporocysts being produced in each oocyst. Four sporozoites develop within each sporocyst. The oocyst wall surrounding the sporocysts is fragile and thin (0.1 μm) and often breaks releasing the sporocysts in the lamina propria. Fully sporulated oocysts or sporocysts are usually shed in the faeces. The sporocysts of *Sarcocystis hirsuta* measure 12 by 8 μm (11 - 14 by 7 - 9 μm), *S. tenella* measure 12 by 8 μm (13 - 14 by 7 - 8 μm) *S. muris*, 10 by 8 μm (9 - 12 by 7 - 9 μm). *Sarcocystis* species of gazelle, 13 by 9 μm (11 - 15 by 8 - 12) (Dubey, 1976). The intermediate host become infected by ingesting sporocysts.

2.5 GENUS *ISOSPORA*

The genus *Isospora* contains two species cyclically transmitted by cats. These are *Isospora felis* and *Isospora rivolta*.

Isospora felis: This has a world wide distribution and is the coccidium most commonly found in the faeces of cats. Its oocysts are easily recognized because they

are large. Cats are the definitive hosts: rodents, dogs and birds may act as intermediate or transport hosts (Frenkel and Dubey, 1972).

Isospora rivolta: This has the second largest coccidian oocysts of the cat. The final host, intermediate host range and the life-cycle is as for *Isospora felis*. Their oocysts measure 25 by 20 μm (Soulsby, 1982).

2.5.1 LIFE-CYCLE

Cats and non-feline hosts become infected by ingesting sporulated oocysts (Shah, 1971; Frenkel and Dubey, 1972). Cats also get infected by ingesting cystic stages in infected intermediate hosts (Frenkel and Dubey, 1972). The sporozoites or bradyzoites penetrate the intestinal epithelium. They multiply extensively producing three generations of schizonts and gametocytes (Shah, 1971). The first generation schizonts measure 11 - 30 by 10 - 23 μm when mature, and contain 16 to 17 banana shaped merozoites, 11 - 15 by 3 - 5 μm. The first generation merozoites enter new host cells, round up and form second generation schizonts (12 - 30 by 10 - 22μm). These form within themselves 2 - 10 or more spindle shaped bodies resembling first generation merozoites in shape and size (Shah, 1971).

The third generation schizonts measure 12 - 16 by 4 - 5 μm each and contain 36 - 70 banana shaped merozoites. The third generation schizonts and merozoites develop within the same host cell and parasitophorous vacuole as the second generation schizonts and merozoites (Shah, 1971). Mature microgametocytes measure 24 - 72 by 18 - 32 μm and contain a central residium. There are large numbers of microgametes which measure 5 - 7 by 0.8 μm, each with two posteriorly directed flagella. The mature macrogametes measure 16 - 22 by 8-13 μm (Shah, 1971).

Oocysts are formed following fertilization of the macrogametes by the microgametes.

The oocysts measure 40 by 30 μm in diameter.

CHAPTER THREE

3. IDENTIFICATION OF THE COCCIDIA OF CATS AROUND KABETE

3.1 SUMMARY

In a preliminary study on the natural coccidia infection in cats, fifty faecal samples were obtained from cats around Kabete and examined for coccidian oocysts. Oocysts of *Besnoitia wallacei*, *Isospora felis*, *Isospora rivolta*, *Sarcocystis muris* and *Toxoplasma gondii* were identified. Rats, mice, rabbits and cats were inoculated with *Toxoplasma* and *Besnoitia* suspects and the characteristic tissue cysts observed.

3.2 INTRODUCTION

The domestic cat is the final host for *Besnoitia besnoiti*, *B. darlingi*, *B. wallacei*, *Isospora felis*, *I.rivolta*, *Hammondia hammondi*, *Sarcocystis hirsuta*, *S. muris*, *S. porcifelis*, *S. tenella*, *Toxoplasma gondii* and some unclassified *Besnoitia* and *Sarcocystis* species (Dubey, 1976).

The coccidia can be identified from the morphology and size of their oocysts and tissue cysts in their intermediate host. However, *Toxoplasma* and *Hammondia* oocysts are morphologically similar and can only be identified by their infectivity to cats and subsequent development in it. *Toxoplasma gondii* oocysts are infectious to cats and can develop in extra-intestinal tissues of the cat (Dubey, 1977)

Information on the species affecting cats in Kenya is lacking. A preliminary study on the natural coccidia infection in cats around Kabete where experimental cats were to be obtained was therefore necessary.

3.3 MATERIALS AND METHODS

3.3.1 CATS

All cats used in the experiment were obtained from around Kabete. They were housed individually in clean cages in the primate unit, College of Agriculture and Veterinary Sciences, University of Nairobi. They were fed on team pet food (Farmers Choice, Nairobi) and water provided *ad libitum*.

Cats were allowed to adopt to caging for a minimum of 21 days, during which faecal samples were collected and examined for coccidian oocysts and helminth infection. They were dewormed after the first week of caging, by oral administration of Ascaten^{R} (Mebendazole USP. 100 mg, Piperazine citrate BP. 275 mg, Bephenium hydroxynaphthoate BPC, 130 mg. (Cosmos Ltd, Nairobi) at a dosage rate of 1 tablet per 5 Kg.

3.3.2 COLLECTION OF FAECAL SAMPLES.

Faecal samples were collected from fifty young and adult cats over a period of four months (July to October, 1990). Trays filled with sand sterilized in an autoclave were placed in each cage (Section 3.3.1) and faecal samples collected from them three times a week during the adaptation period and daily for at least 30 days post-infection. Faecal containers (Polypots) were also distributed to homes around Kabete where 20 samples were collected.

3.3.3 PROCESSING OF FAECAL SAMPLES.

Faecal samples were processed as described by Dubey, Swan and Frenkel, (1972) with minor modifications. They were processed on the day of collection or stored at 4°C. They were soaked in water until soft. Excess water was drained off directly into a sink and flashed off (not in sterilizable containers).

Faeces were emulsified into a thick paste and suspended in about 10 volumes of sucrose solution of specific gravity 1.15 (40% w/v of sucrose in water). The suspension was sieved through tea strainers to remove large particles. This was then centrifuged at 1,000 revolution per minute (r.p.m.) in a floating head type centrifuge for 10 minutes.

From the supernant 0.5 ml was aspirated with a 1 ml Pasteur pipette and rubber bulb. A drop was placed on a microscope slide, a 22 by 22 mm cover slip placed on top, allowed to stand for 5 minutes and examined for coccidian oocysts.

Recovered oocysts were sporulated by mixing 0.5 ml of the supernant with 5 ml of 2% aqueous sulphuric acid. The mixture was poured into a petri-dish to a depth of 0.5 cm, and incubated at room temperature (20-26°C) with constant aeration until sporulation was complete. The sporulated oocysts were stored in 2% sulphuric acid at 4°C.

Before oral inoculation of the sporulated oocysts into test animals the acid was diluted with water and centrifuged at least three times. Oocyst numbers were estimated using a haemocytometer, and diluted to the required doses.

3.3.4 OOCYSTS MORPHOLOGY AND SIZES

Oocysts were identified by their sizes and morphological characteristics as described by Wallace and Frenkel (1975), Dubey (1976), Frenkel (1977) and McKenna and Charleston (1980b) and Soulsby (1982).

3.3.5 MEASUREMENTS (MICROMETRY)

Measurements of oocysts, tissue cysts and *Besnoitia* developmental stages were taken at objective x10 or x40 of a light microscope. This was achieved by use of a micrometer eye-piece calibrated as described by Garcia and Ash (1979).

3.3.6 ANIMAL INOCULATION

For further identification of oocysts suspected to be of *Toxoplasma* and *Besnoitia*, laboratory animals (mice, rats and rabbits) and cats were inoculated (Figs. 3.1, 3.2). Mice, rats and rabbits used in the experiments were obtained from the small animal unit in the Department of Veterinary Pathology and Microbiology, University of Nairobi and maintained on commercial mice pencils and rabbit pellets.

Seven cats used in the oocysts identification were divided into three groups (Group A-C). Group A consisted of three members, while Groups B and C had two members each.

3.3.6.1 *TOXOPLASMA* AND *BESNOITIA* MIXTURE

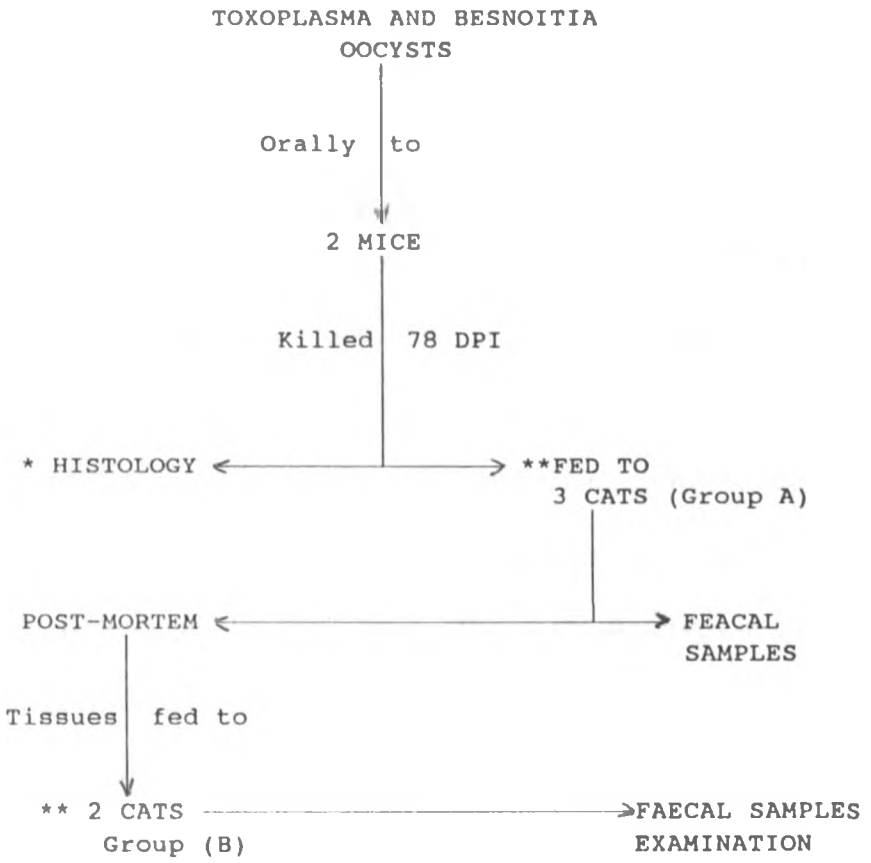
A mixture of *Toxoplasma* and *Besnoitia* oocysts recovered from one of the cat faecal samples was inoculated into test animals. Two mice were orally inoculated with approximately 1 x 10^4 of the mixture of the sporulated oocysts. They were killed on

day 78 post-infection. Small tissue samples 0.5 - 2 cm long were taken from the intestines and spinal cord and blocks 1 - 2cm^3 from the liver, spleen, skeletal muscles, kidneys, lungs, heart, and brain. They were preserved in 10% formalin, embedded in paraffin wax, section at 6μm thickness and stained with haematoxylin and eosin (H&E). A few selected sections were stained with periodic acid schiff (PAS) for identification of *Besnoitia* cysts.

The rest of the mice carcasses were fed to three cats (Group A). Faecal samples from the cats were collected daily for 43 days post-infection and examined for coccidian oocysts. They were then killed 43 days post-infection. Small tissue samples were taken from the intestines, liver, kidneys, skeletal muscles, lungs, heart and spinal cord. They were preserved in 10% formalin and processed for histological examination.

To test for the extra-intestinal development and infectivity of these tissues to other cats, pieces of skeletal muscles, liver, lungs and brain from these cats (Group A) were fed to two cats (Group B). Faecal samples were collected from Group B cats for 30 days post-infection and examined for coccidian oocysts.

Figure 3.1 Further identification of oocysts suspected to be of Toxoplasma and Besnoitia.



KEY: * Differentiation of Besnoitia tissue cysts from Toxoplasma/Hammondia.

** Differentiation between Toxoplasma and Hammondia

3.3.6.2 *BESNOITIA* TYPE OOCYSTS

Besnoitia type oocysts recovered from one of the cat faecal samples were orally infected to twelve rats, thirty two mice and two rabbits for further identification (Fig. 3.2). Each of the test animals was orally infected with approximately 1 x 10^6 sporulated oocysts. Two rats, two mice and one rabbit were kept as controls.

Fifteen of the infected mice were serially sacrificed at two day intervals to determine the time taken for tissue cysts to be grossly visible. The rats, rabbits, control mice and a seventeen of the inoculated mice that remained after the serial sacrifice were killed 78 days post infection, and examined for *Besnoitia* tissue cysts. Small tissue samples were taken from the intestines, liver, spleen, tongue, brain, spinal cord, skeletal muscles and kidneys. They were preserved in 10% formalin and processed for histological examination.

The rest of the infected rat and mice carcasses were fed to two cats (Group C) and 13 others (Chapter 4). Faecal samples from cats in Group C were collected daily for 45 days post-infection, processed and examined for *Besnoitia* oocysts.

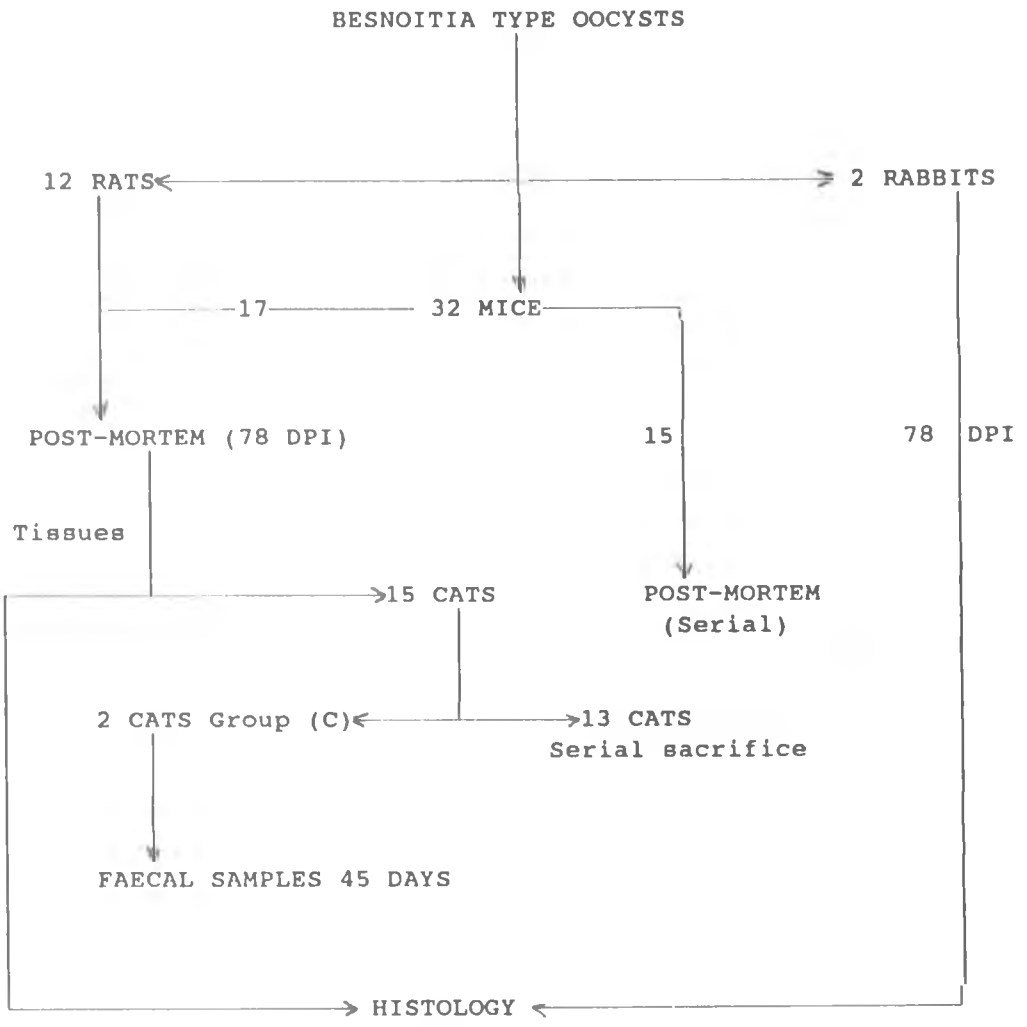


Figure 3.2 Further identification of Besnoitia type oocysts recovered from cat faeces.

3.4 RESULTS

3.4.1 OOCYST MORPHOLOGY AND SIZES

From cat faecal samples around Kabete, five different types of coccidian oocysts were recovered and identified as described below. Of the 50 faecal samples 19 (38%) had only one type of oocysts, 23 (46%) had mixtures of oocyst types, while 8 (16%) were free of coccidian oocysts (Table 3.1).

TABLE 3.1 "Occurrence, identification and sizes of unsporulated coccidian oocysts in faecal samples from cats around Kabete".

Coccidian species	Frequency %	Size in micrometers L	W
Isospora felis	22	42.4 ± 3.3 x 34 ± 2.1 (34.4 - 48.4 x 29.3 - 38.5)	
Isospora rivolta	10	25.2 ±2.4 x 23.2 ± 2.5 (20.7 - 29.5 x 16.7 - 28.0)	
Sarcocystis muris	4	11.0 ± 0.8 x 7.9 ± 0.5 (9.7- 13.3 x 7.1 -9.5)	
Besnoitia wallacei	2	16.6 ± 1.6 x 14.5 ± 1.0 (14.0 -19.4 x 12.6 - 17.8)	
I.felis + I. rivolta	34		
I. felis + Toxoplasma gondii	10	12.0 ± 0.8 x 10.3 ± 0.7* (10.1 - 13.5 x 8.9 - 12.2)*	
B.wallacei + T. gondii	2		
No Coccidian oocysts	16		

Key
L: Length
W: Width.
* *Toxoplasma gondii* oocysts only
Values in parenthesis represent the range

3.4.1.1 *Isospora felis*

The unsporulated oocysts of *I. felis* were spherical to ovoid in shape. The sporont at the time of shedding was

usually shrunken. The oocyst wall was smooth and bilayered. Micropyle and polar granules were absent. These were the largest recovered oocysts and measured on average 42.4 ± 3.3 by 34.0 ± 2.1 μm (n = 50) with a range of 34.4 - 48.4 by 29.3 - 38.5 μm.

The sporulation time was 1 to 3 days at room temperature (20 - 26°C). The sporulated oocysts were ovoid in shape (Fig. 3.3). On average, they measured 42.8 ± 4.1 by 34.0 ± 2.4 μm (n = 50) with a range of 29.3 - 50.1 by 28.0 - 38.1 μm. They contained two sporocysts which were ellipsoidal. On average the sporocysts measured 19.9 ± 1.7 by 18.1 ± 1.2 μm (n = 50) with a range of 15.8 - 23.0 by 15.0 - 21.3 μm. The oocyst residium was absent. Each sporocyst contained four sporozoites and sporocyst residium.

3.4.1.2 *Isospora rivolta*

The unsporulated oocysts of *I. rivolta* were subspherical to ovoid in shape (Fig. 3.3). They had a smooth bilayered wall. They lacked polar granules. These were the second largest oocysts recovered. On average, they measured 25.2 ± 2.4 by 23.2 ± 2.5 μm (n = 50), with a range of 20.7 - 29.5 by 16.7 - 28.0 μm.

The sporulation time was 4 - 7 days at room temperature. The sporulated oocysts were subspherical to ovoid. On average they measured 24.6 ± 2.3 by 23.1 ± 2.2 μm (n = 50), with a range of 20.7 - 28.3 by 18.9 - 26.5 μm.

They contained two sporocysts which measured on average 12.2 ± 1.3 by 9.7 ± 0.5 μm (n = 100), with a range of 10.2 - 15.3 by 8.9 - 11.0 μm. Oocyst residium was absent. Each sporocyst contained four sporozoites and sporocyst residium.

3.4.2 *Sarcocystis muris*

The free sporocysts of *Sarcocystis muris* were ellipsoidal (Fig. 3.4). They contained four sporozoites and a granular sporocyst residium. On average they measured 11.0 ± 0.8 by 7.9 ± 0.5 μm (n = 50), with a range of 9.7 - 13.3 μm by 7.1 - 9.5 μm.

3.4.3 *Toxoplasma gondii*

The unsporulated oocysts of *Toxoplasma gondii* were spherical to subspherical (Fig.3.5). The sporont at time of shedding almost filled the oocysts, but later shrunk. The oocyst wall was colourless and bilayered. Micropyle and polar granules were absent. On average they measured 12.0 ± 0.9 by 10.3 ± 0.7 μm (n = 50), with a range of 10.1 - 13.5 by 8.9 - 12.2 μm.

The sporulation time was 3 - 7 days at room temperature. The sporulated oocysts were subspherical to ellipsoidal. They contained two sporocysts. Oocyst residium was absent. On average they measured 12.4 ± 0.8 by 10.4 ± 0.6 μm (n=50), with a range of 10.5 - 13.4 by 9.5 - 11.3 μm. The sporocysts were ellipsoidal and on average measured 8.0 ± 0.3 by 6.1 ± 0.5 μm (n = 50), with

a range of 6.9 - 8.8 by 5.2 - 7.1 μm. The sporocyst contained four sporozoites and a sporocyst residium.

3.4.4 *Besnoitia wallacei*

The unsporulated oocysts of *Besnoitia wallacei* were spherical to ellipsoidal (Figs 3.6, 3.7). The sporont at the time of shedding almost filled the oocyst. The wall was colourless, bilayered and smooth. Micropyle and polar granules were absent. On average, they measured 16.6 ± 1.6 by 14.5 ± 1.0 μm (n = 50), with a range of 14.0 - 19.4 by 12.6 - 17.8 μm.

The sporulation time was 4 - 7 days at room temperature. The sporulated oocysts were spherical to ellipsoidal and contained two sporocysts each with four sporozoites (Fig 3.7). Oocysts residium was absent. On average, they measured 17.3 ±1.5 by 13.9 ± 0.9 μm (n = 50), with a range of 14.4 - 20 by 12.0 - 16.2 μm. The sporocysts were ellipsoidal. On average they measured 10.4 ± 1.4 by 8.3 ± 0.9 μm (n = 100), with a range of 7.6 - 13.8 by 6.5 - 10.8 μm. Each sporocyst contained four sporozoites and a diffuse sporocyst residium. The stieda body was absent.

3.5 ANIMAL INOCULATION

3.5.1 *TOXOPLASMA* AND *BESNOITIA* MIXTURE

The two mice (Section 3.3.6.1) that were orally inoculated with a mixture of *Toxoplasma* and *Besnoitia* oocysts had numerous grossly visible cysts on the serosal

surface of the caecum and ileum. These were white, milliary and sand-like. Fewer cysts were seen on the anterior colon, jejunum, duodenum, mesentery, omentum and the heart.

Microscopically, spherical to ovoid *Besnoitia* cysts were seen in tissue sections of lungs (Fig. 3.8), heart, kidneys (Fig.3.9), mesentery and intestines (Figs. 3.10; 3.11). They measured 208.5 ± 28.5 by 164.1 ± 36.4 μm (n = 25) with a range of 121.9 - 255.3 by 80.5 - 210.3 μm. The cyst wall was thick (Fig. 3.12) and of varying thickness with an average of 7.1 μm (2.5 - 24.9 μm). The wall was PAS positive, and the host cell nuclei were hypertrophic and hyperplastic. There was little or no inflammatory reaction accompanying the cysts. The few inflammatory cells involved were mainly lymphocytes and macrophages.

Toxoplasma cysts were seen in sections of the brain only (Fig. 3.13). They were spherical to subspherical in shape and on average measured 22.9 by 18.6 μm (n = 20).

The three cats in Group A produced both *Toxoplasma* and *Besnoitia* oocysts after ingesting infected mice carcasses. The prepatent period for *Toxoplasma* was 9 to 10 days and patency lasted 10 to 15 days. The prepatent period for *Besnoitia* was 12 to 17 days, while patency lasted 15 to 18 days. There were no gross or histological lesions in the intestinal sections suggesting parasite infections in the three cats.

The two cats (Group B) fed on extra-intestinal tissues of Group A cats produced *Toxoplasma* oocysts. The prepatent

and patent periods were 9 to 10 and 10 to 15 days respectively as for Group A cats. *Besnoitia* oocysts were however not recovered from these cats.

3.5.2 *BESNOITIA TYPE OOCYSTS*

In mice, cysts on serosal surfaces of the intestines became grossly visible on day 28 of infection. The cysts in mice and rat tissues were grossly and histologically similar to those described in section 3.5.1

In rabbits scleral conjunctival cysts became grossly visible on day 36 of infection. At post-mortem, cysts were seen in the subcutis, fascia, skeletal muscles, in the peritoneum, mesentery, omentum, on the serosal surfaces of the intestines, stomach, gall bladder and urinary bladder, on the capsules of the liver and kidney. Few cysts were seen on the diaphragm and mucosal surfaces of the oesophagus, larynx and trachea. Microscopically, cysts were seen in the skin and tongue sections. No cysts were seen in tissues of all control animals.

The two cats in Group C produced *Besnoitia* type oocysts between day 12 and 30 post-infection.

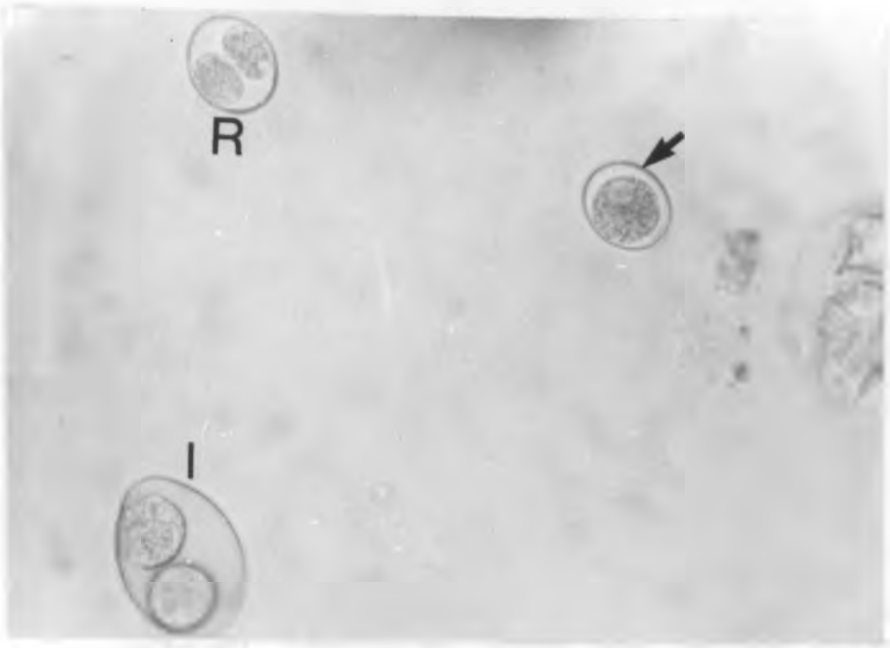


Figure 3.3: Oocysts of *Isospora* species recovered from faeces of a naturally infected cat. *I. felis* (I)and *I.rivolta* (R) each with two sporoblasts and unsporulated oocyst of *I.rivolta* (arrow). Unstained x400.

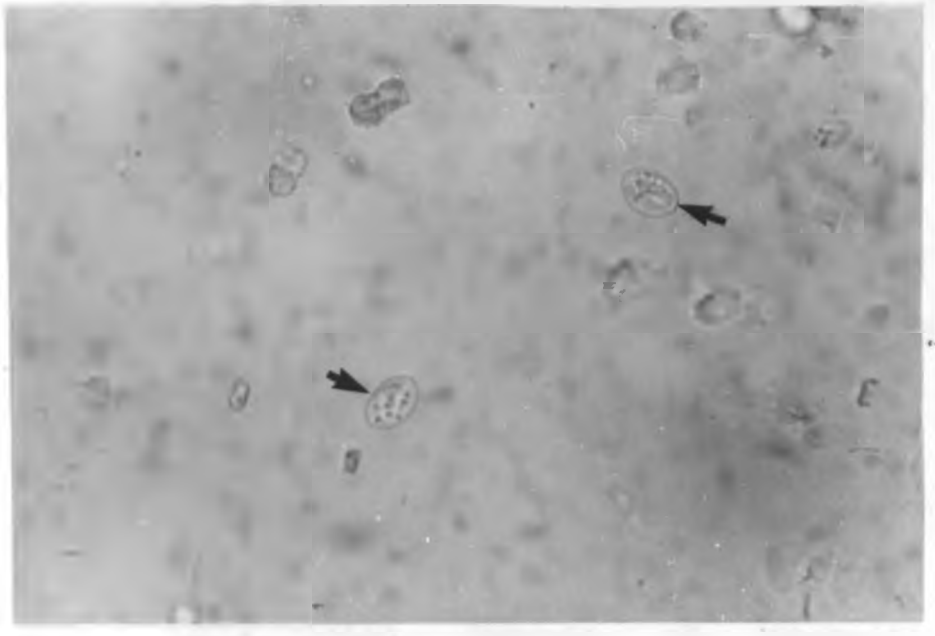


Figure 3.4: Free sporocysts of *Sarcocystis muris* (arrows) recovered from faeces of a naturally infected cat. Unstained x630.

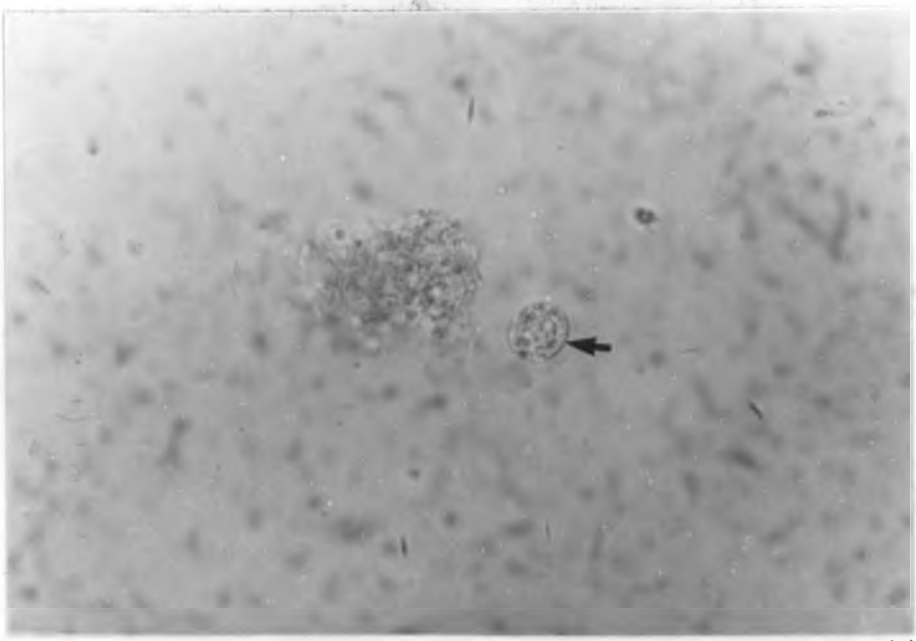


Figure 3.5: Unsporulated oocyst of *Toxoplasma gondii* (arrow) recovered from the faeces of a naturally infected cat. Unstained x630.

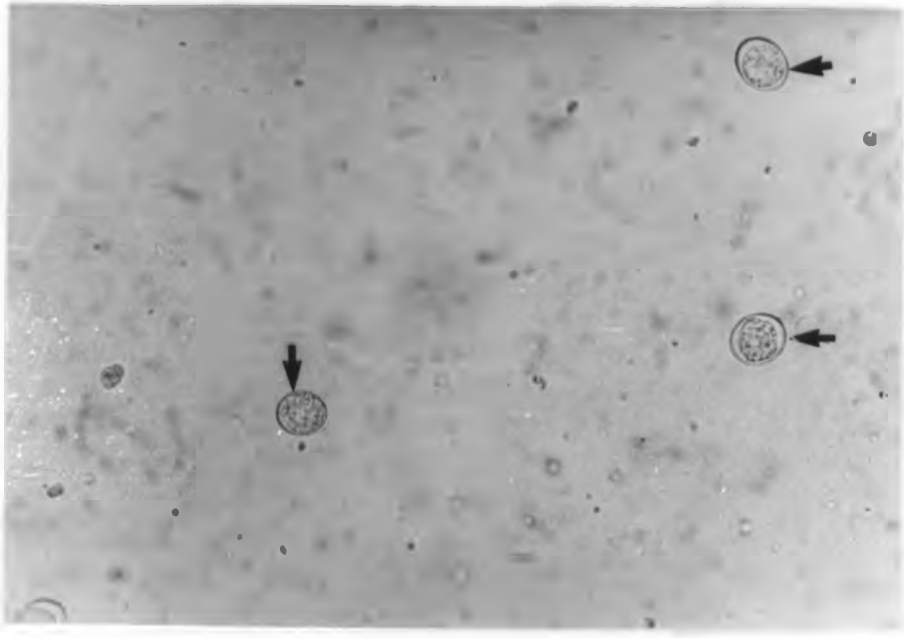


Figure 3.6: Unsporulated oocysts of *Besnoitia walllacei* (arrows) recovered from the faeces of a naturally infected cat. Unstained x400.

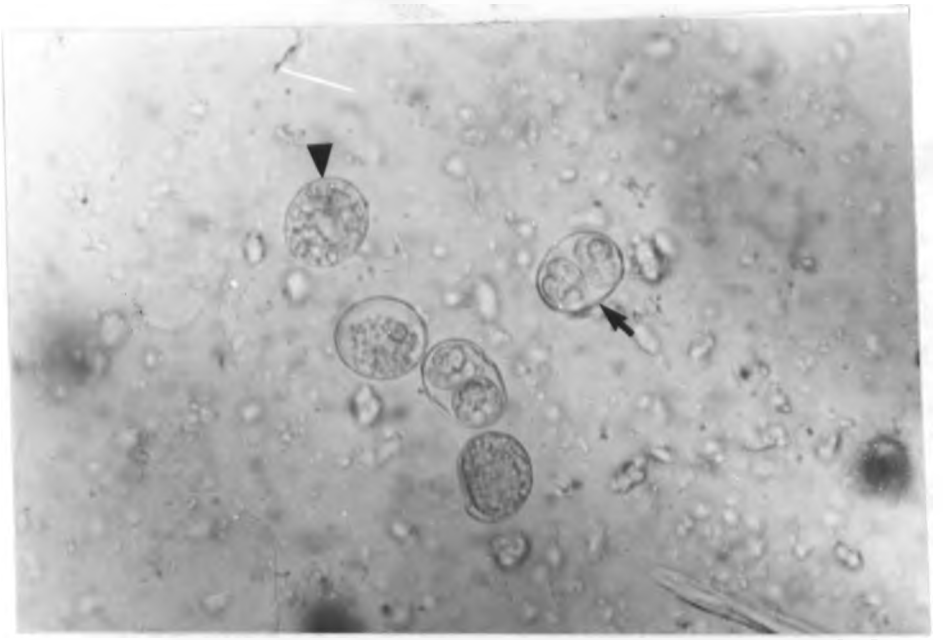


Figure 3.7: *Besnoitia wallacei* oocysts recovered from faeces of a naturally infected cat. Unsporulated oocyst (arrow head) and sporulated oocysts (arrow) with two sporocysts, each with four sporozoites. Unstained x630.

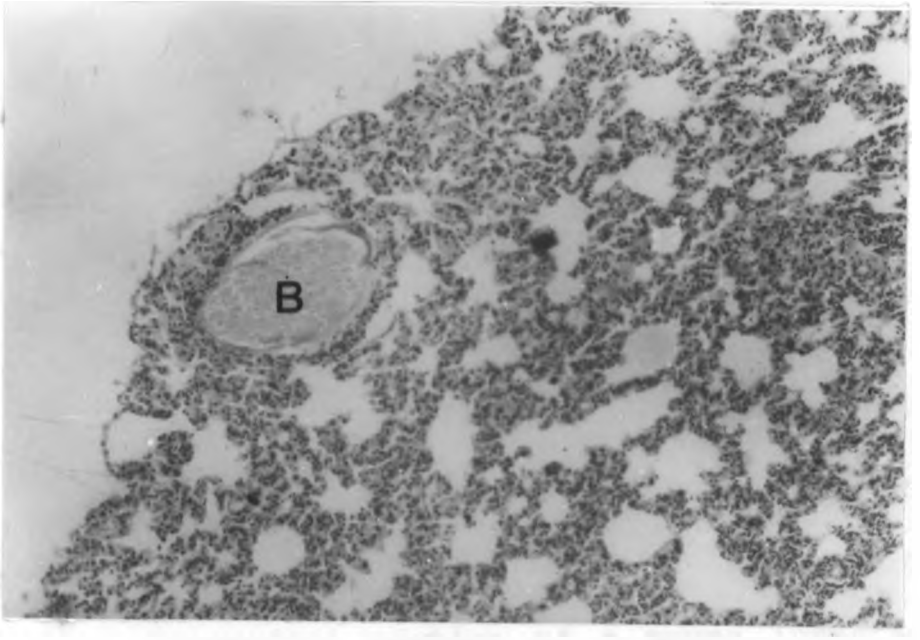


Figure 3.8: Lung of a mouse infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts 78 days post-infection. Note the *Besnoitia* cyst (B). H&E x100.

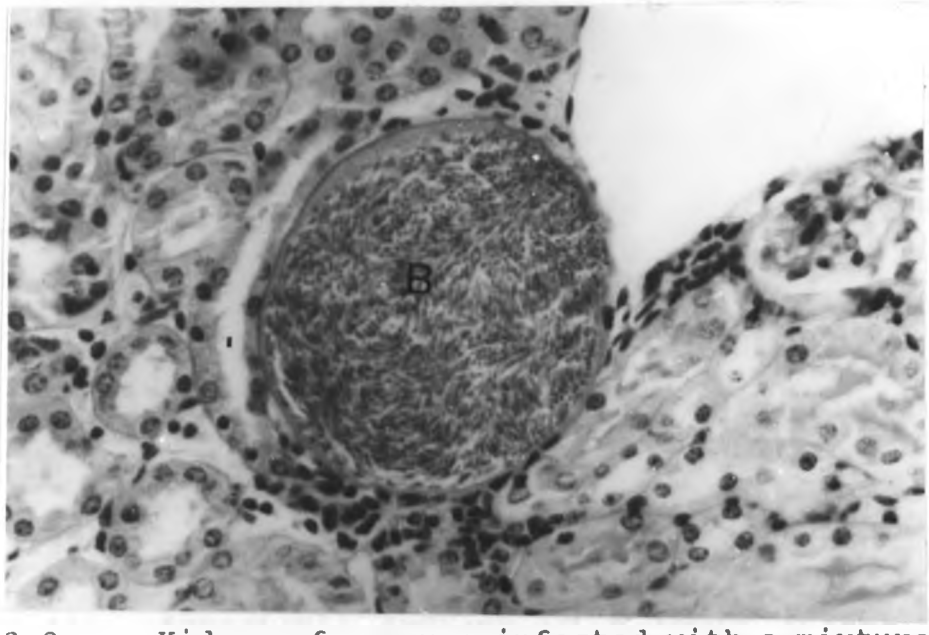


Figure 3.9: Kidney of a mouse infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts 78 days post-infection, showing a *Besnoitia* cysts (B) containing numerous bradyzoites. H&E x400.

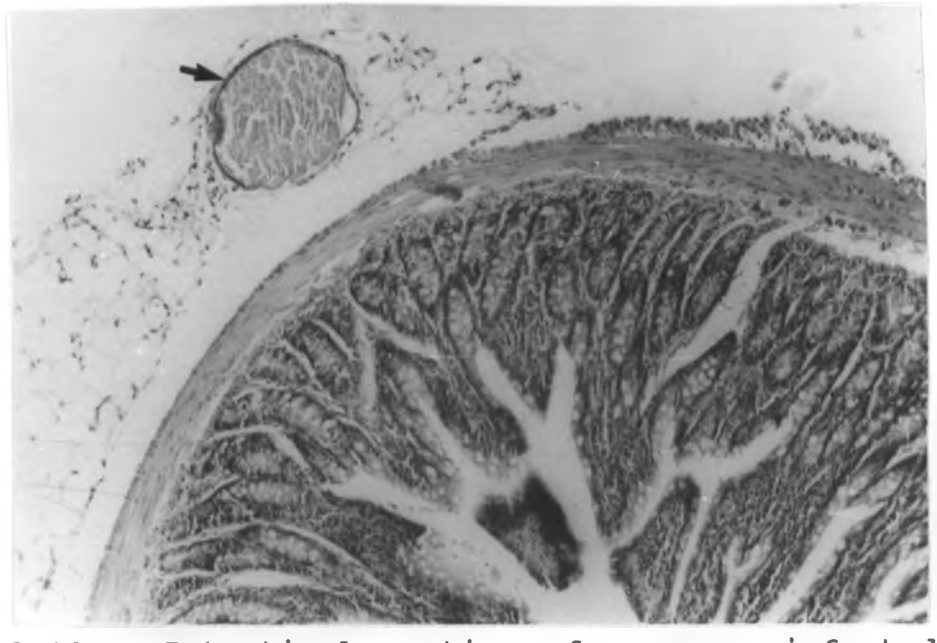


Figure 3.10: Intestinal section of a mouse infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts 78 days post-infection. A *Besnoitia* cyst is present in the mesentery (arrow). H&E x100.

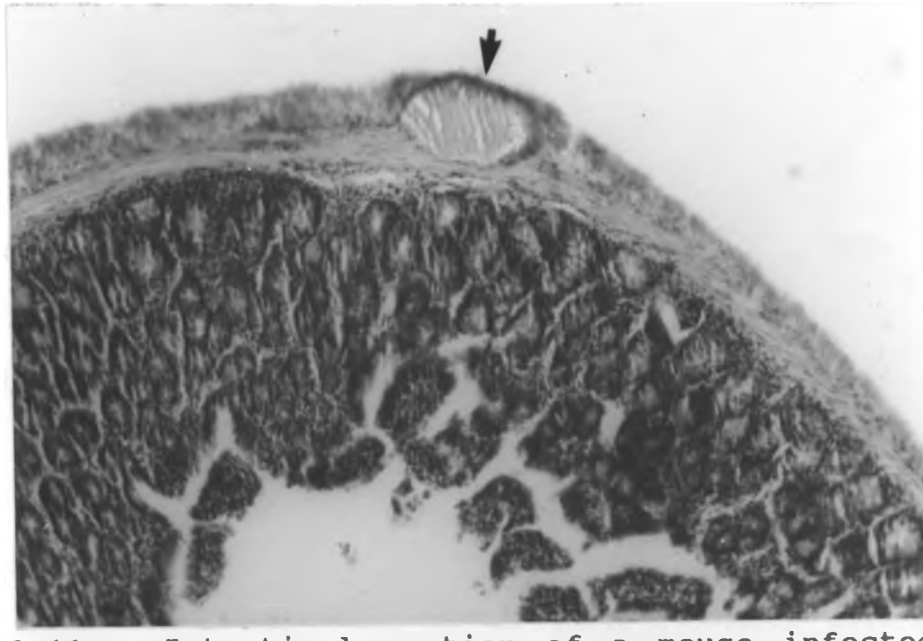


Figure 3.11: Intestinal section of a mouse infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts 78 days post-infection. Note the *Besnoitia* cyst in the tunica muscularis (arrow) causing a bulge on the serosal surface. H&E x100.

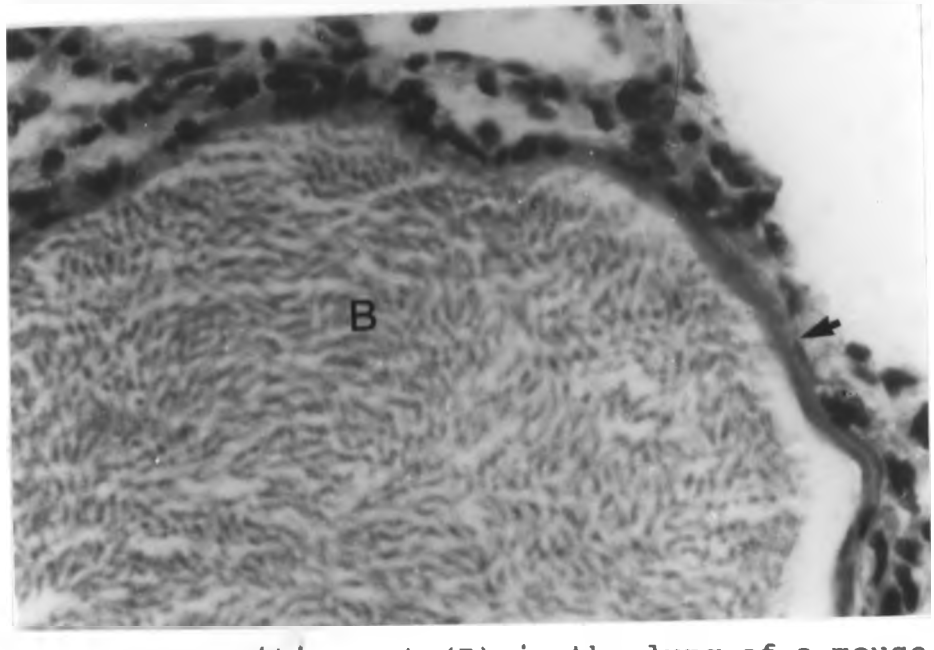


Figure 3.12: *Besnoitia* cyst (B) in the lung of a mouse showing the thick cyst wall (arrow). Note the numerous bradyzoites in the cyst. PAS x630.

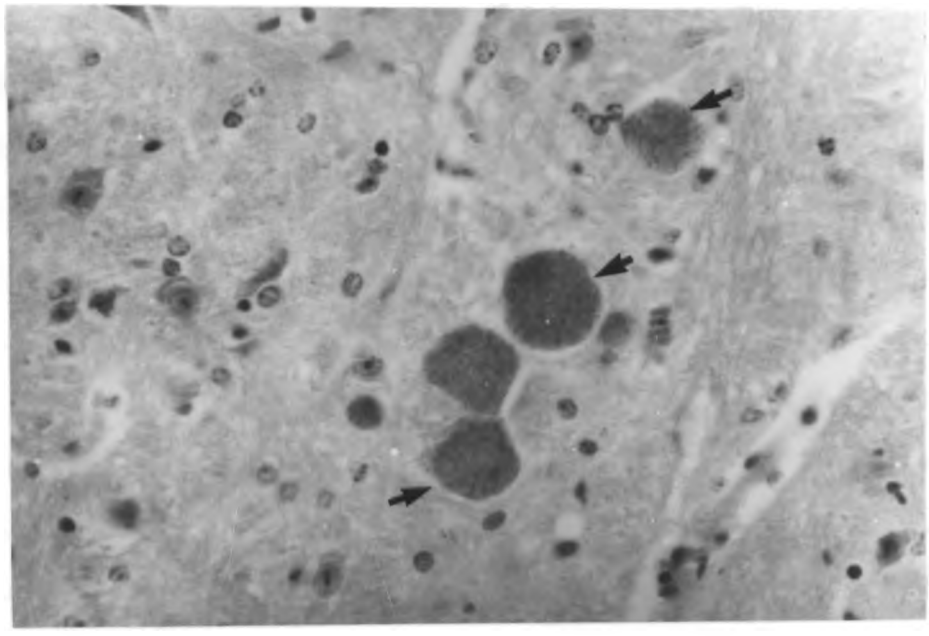


Figure 3.13: Brain section of a mouse infected with a mixture of *Toxoplasma* and *Besnoitia* oocysts 78 days post-infection, showing *Toxoplasma* cysts (arrows). H&E x400

3.6 DISCUSSION

Representatives of four of the five known genera of coccidia of the cat were recovered from faecal samples. This included *Isospora*, *Sarcocystis*, *Toxoplasma* and *Besnoitia*. Further investigations involving bigger samples may possibly reveal the presence of *Hammondia*.

Members of the genus *Isospora* have a world wide distribution. They have a wide range of final hosts including man, dogs and cats (Dubey, 1977). The cat is the final host for *Isospora felis* and *I.rivolta*. In this study, *I. felis* was the most commonly encountered coccidium of the cat. It's oocysts were easily identified because of their large size. *Isospora rivolta* was the second both in the frequency of occurrence and size.

Sarcocystis was originally described from the muscles of pigs. It was later found to be a common parasite in the musculature of reptiles, birds, small rodents and herbivores (Dubey, 1976). More than one species may parasitize a single intermediate host and a single species can develop in several final hosts (Heydorn *et al*, 1975). In the present study, *Sarcocystis* sporocysts were recovered in 4% of the cat faecal samples examined. They measured 11 by 7.9 μm (9.7 - 13.3 by 7.1 - 9.5 μm). The measurements were therefore similar to those of *Sarcocystis muris* (9 - 12 by 7 - 9 μm) described by Dubey (1977).

Toxoplasma is morphologically similar to *Hammondia*. However, unlike *Hammondia*, *Toxoplasma* oocysts are infectious to cats and also develops in extra-intestinal tissues of the cat. Therefore the production of oocysts by cats fed on extra-intestinal tissues of other cats in the present study (section 4.3.4) confirms the presence of *Toxoplasma* around Kabete.

Information on toxoplasmosis in man and animals in Kenya is scanty. However, human and monkey infection have been reported (Bakal, *et al.*, 1968; Olubayo and Mwongela, 1978). The recovery of *Toxoplasma* oocysts from cat faeces in the present study is an important addition.

Currently, at least 6 species of *Besnoitia* are recognised. Their geographical distribution has been reviewed by Frenkel (1977). In Kenya, *Besnoitia* infections have been reported in cattle, horses and goats (Bwangamoi, 1967, 1968, 1972; Bwangamoi *et al.*, 1989). In the present study, *Besnoitia* oocysts were recovered in cat faeces for the first time in Kenya. This was an important finding due to the implication of the cat in the cyclic transmission of *Besnoitia* species.

The biological and morphological characteristics of the current isolate were similar to those of *Besnoitia wallacei* reported by Wallace and Frenkel (1975); Frenkel (1977); Ito *et al* (1978) and McKenna and Charleston (1980b). The unsporulated oocysts were spherical to ellipsoidal and measured 16.6 by 14.5 μm on the average. The sporulation time was 4 - 7 days at room temperature (20-26°C). The sporulated oocysts were

infectious to rats, mice and rabbits where they resulted in tissue cyst formation. The tissue cysts in mice became grossly visible on day 28 of infection. Similar observations were made by Wallace and Frenkel (1975). Microscopically, the tissue cysts were spherical to ovoid and measured 208.5 by 164.1 μm. The cyst wall was thick and stained positively with periodic acid schiff (PAS). The host cell nuclei were hypertrophic and hyperplastic. The host cell cytoplasm and nuclei were pushed peripherally to form part of the cyst wall. These are important characteristics in the identification of *Besnoitia* species.

CHAPTER FOUR

4 A STUDY OF THE DEVELOPMENT OF BESNOITIA WALLACEI IN THE CAT

4.1 SUMMARY

The development of *Besnoitia wallacei* in the cat was studied. Thirteen cats fed on tissues of mice and rats infected with *Besnoitia wallacei* were serially sacrificed between day 1 and 16 of infection. Histological sections of the liver and intestines were examined. Asexual and sexual stages were observed between day 6 and 16 post-infection. Schizonts were seen in epithelial cells and lamina propria of the intestines and in the liver. Mature microschizonts in epithelial cells measured 22.6 by 14.7 μm (15.4 - 30 by 10.6 - 24.0 μm). Macroschizonts in the lamina propria measured 66.6 by 50.3 μm (n = 25) with a range of 50 - 90 by 33.6 - 76.6 μm. Those in the liver measured 70.9 by 55.0 μm (n = 5).

Sexual stages were seen in epithelial cells of the small intestines only. Macrogametocytes measured 11.4 by 8.3 μm (9.1 - 14.4 by 5.0 - 14.2 μm). Macrogametes measured 12.9 by 8.8 μm (10.1 - 15.6 by 7.2 - 9.6 μm). Microgametocytes measured 10.9 by 8.4 μm (9.1 - 14.0 by 6.2 - 11.8 μm).

4.2 INTRODUCTION

The cat is involved in the cyclic transmission of *Besnoitia besnoiti* (Peteshev *et al.*, 1974), *B. darlingi* (Smith and Frenkel, 1977, 1984) and *B.wallacei* (Wallace and Frenkel, 1975, Frenkel, 1977) as demonstrated by oocyst production. The oocysts morphology is well described by these authors. The biological characteristics of the species is well summarized by McKenna and Charleston (1980b). Little is known of the endogenous development of *B. besnoiti* and *B. darlingi* in the cat.

Endogenous stages of *Besnoitia wallacei* in the cat have been described by Wallace and Frenkel (1975) and Frenkel (1977) for the U.S.A isolate and Ito *et al* 1978 for the Japanese isolate. However, there were differences in size of macroschizonts (giant schizonts) described by Frenkel (1977) who reported them as being 500-800 μm while those of Ito *et al* (1978) were 50-120 by 35-80 μm. There is need to study and compare the endogenous stages of the Kenyan isolate with those described by other workers.

4.3 MATERIALS AND METHODS

Thirteen 2-3 month old kittens were fed tissues from rats and mice previously infected with sporulated *Besnoitia wallacei* oocysts (Chapter Three, section 3.3.6.2). Four of the cats were sacrificed daily for the first four days. The remaining nine cats were sacrificed individually at two day

intervals until day 10 of infection subsequent to which two cats were sacrificed on day 12, 14 and 16. Small tissue samples 2 - 3 cm long were taken from the duodenum, jejunum, ileum, colon and blocks 1 - 2cm^3 from the liver, fixed in 10% formalin and processed for histological examination. Faecal samples from remaining cats were examined daily for *Besnoitia* oocysts, until the last cat was killed on day 16.

4.4 RESULTS

4.4.1 FAECAL SAMPLES

Besnoitia wallacei oocysts were recovered from cat faeces on days 12 to day 16 of infection. The oocysts were morphologically similar to those described in Chapter 3 (Section 3.4.4).

4.4.2 ENDOGENOUS STAGES

The first endogenous stages were seen on day 6 of infection. These were microschizonts found in epithelial cells and young macroschizonts in the lamina propria of the small intestines and in the liver. Microschizonts in epithelial cells (Fig. 4.1) were seen between day 6 and 16 of infection. They were oval in shape and greatly distended the infected cells. On day 10 they had a mean size of 22.6 by 14.7 μm (n = 15) with a range of 15.4 - 30 by 10.6 - 24 μm.

Subspherical to ellipsoidal macroschizonts were seen in the lamina propria between day 6 and 16 of infection

(Figs.4.2; 4.3). They were closely associated with blood vessels, but the parasitized cell type could not be determined. From day 6 of infection there was an increase in number and size of the macroschizonts from 14.7 by 11.2 μm (n = 2) (range 14.4 - 14.9 by 8.9 - 13.4 μm) to 66.6 by 50.3 μm (n = 25) (range 53 - 90 by 33.6 - 76.6 μm) on day 12 to 16 of infection. The schizonts in lamina propria were the predominant asexual stages seen between day 12 and 16 post-infection.

Macroschizonts in the liver were seen on day 6 and 12 post-infection (Fig. 4.4). They were closely associated with sinusoids and on average measured 70.9 by 55.0 μm (n = 5) with a range of 64.1 - 89.3 by 50.9 - 63.8 μm.

Sexual stages were seen between day 10 and 16 of infection. They were located in the goblet cells between the nuclei and vacuoles. Macrogametocytes (Fig. 4.5) were the most numerous of the sexual stages seen. They measured on the average 11.4 by 8.3 μm (n = 15) with a range of 9.1 - 14.4 by 5.0 - 14.2 μm. Macrogametes (Fig. 4.6) had large centrally placed nuclei and were bigger than macrogametocytes and measured 12.9 by 8.8 μm (n = 10) with a range of 10.1 - 15.6 by 7.2 - 9.6 μm. Few microgametocytes were seen on day 16 of infection. They measured 10.9 by 8.4 μm (n = 5) with a range of 9.1 - 14 by 6.2 - 11.8 μm.

Oocysts at various developmental stages were seen between day 10 and 16 of infection. They were either in epithelial

cells or extruded into the intestinal lumen (Fig. 4.7). The extruded oocysts were spherical to ellipsoidal and contained granular cytoplasm that completely filled their cavities and had a clear unstained wall. They measured on average 15.2 by 14.1 μm (n = 10). All endogenous stages seen in the intestines were located between the duodenum and the ileum. They were most numerous in the ileum. Extruded oocysts were the only developmental stage seen in the colon.

4.4.3 PATHOLOGY

There was marked loss of epithelial cells between day 10 and 16 of infection. Desquamated epithelial cells with various endogenous stages, from schizonts to oocysts were seen. There was a mild infiltration by the inflammatory cells into the lamina propria of the small intestines. The cells were mostly lymphocytes and plasma cell, with a few eosinophils, neutrophils and macrophages. In the liver, there were a few necrotic foci. These foci were infiltrated by neutrophils, lymphocytes, macrophages and a few eosinophils.

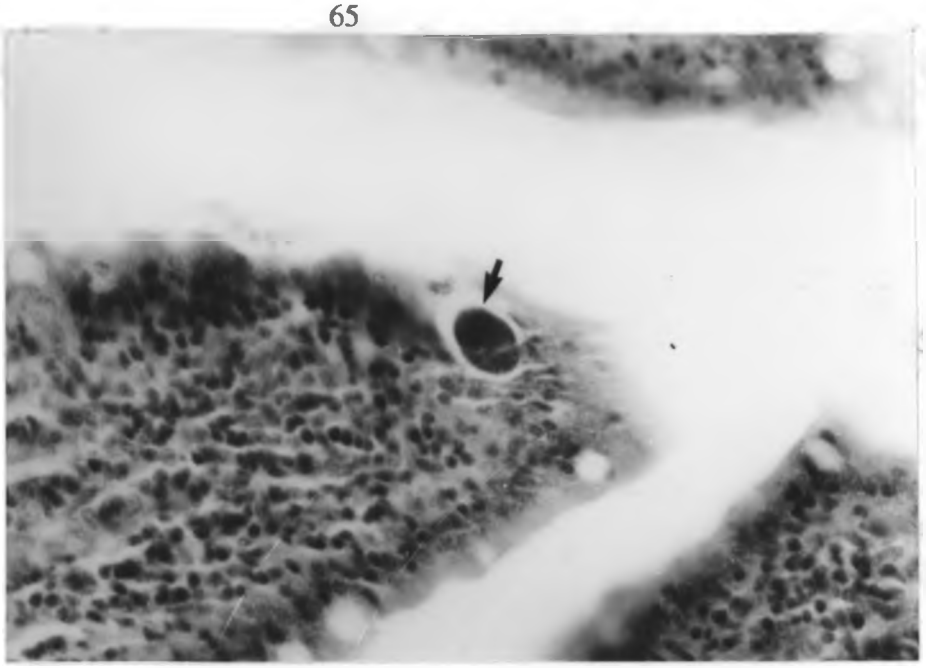


Figure 4.1: Microschizont of *Besnoitia wallacei* in the epithelial cell (arrow) of the ileum of a cat 8 days post-infection. H&E x400.

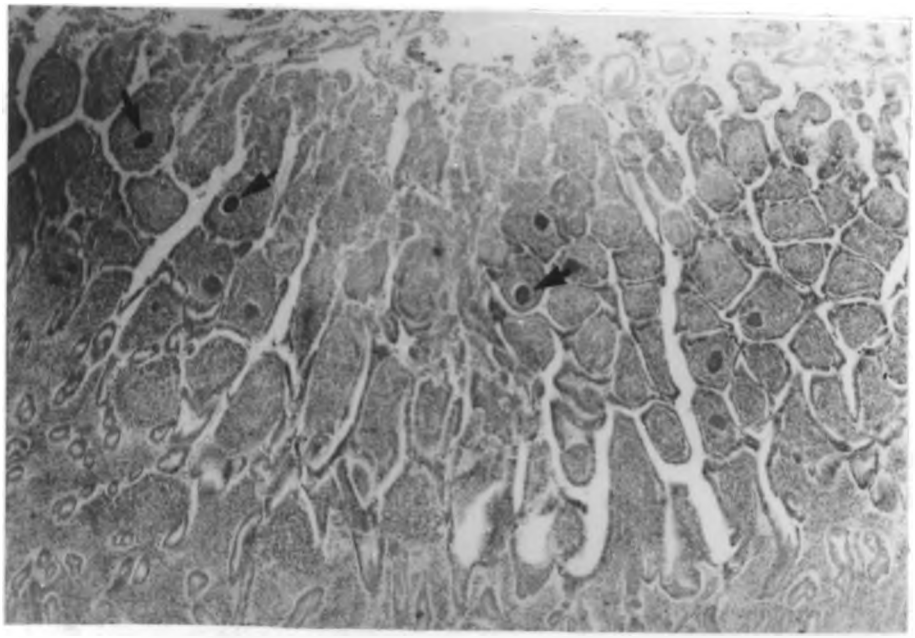


Figure 4.2: Macroschizonts of *B.wallacei* in the lamina propria (arrows) of the ileum of a cat 12 days post-infection. Note the desquamation of the epithelium. H&E x40.

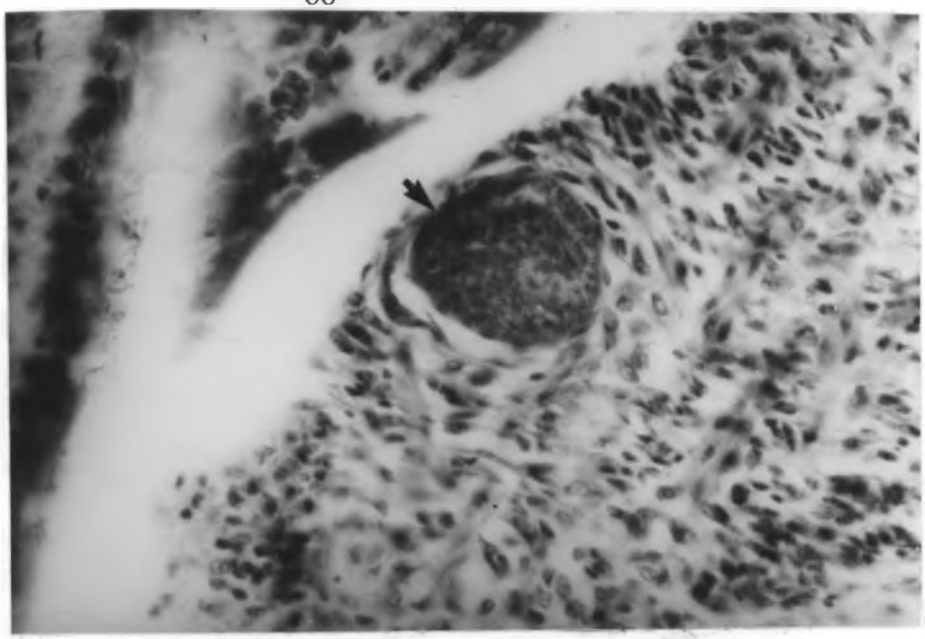


Figure 4.3: Macroschizont of *B. wallacei* in the lamina propria (arrow) of the ileum of a cat 12 days post-infection. H&E x400.

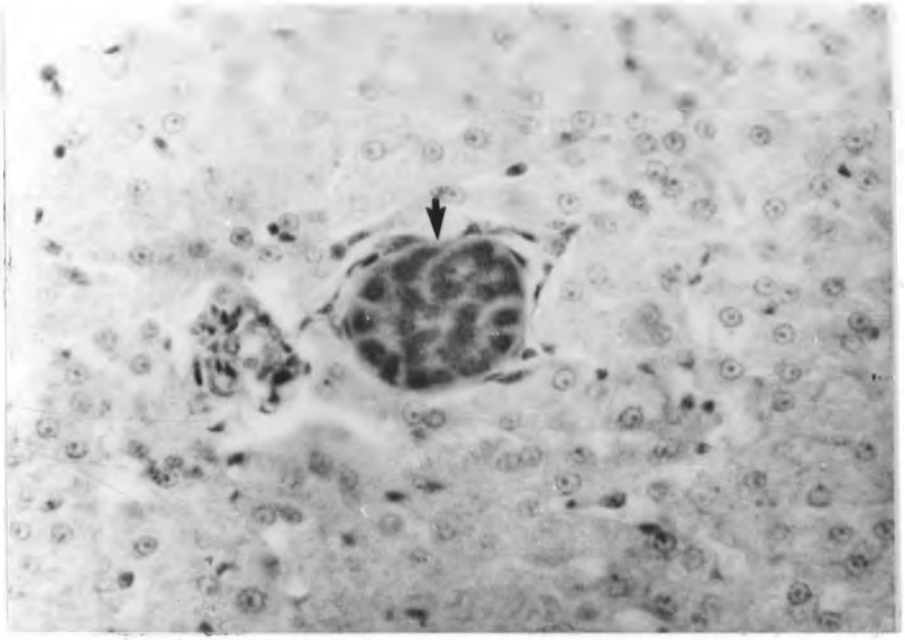


Figure 4.4: Macroschizonts of *B. wallacei* in the liver (arrow) of a cat 12 days post-infection. H&E x400.

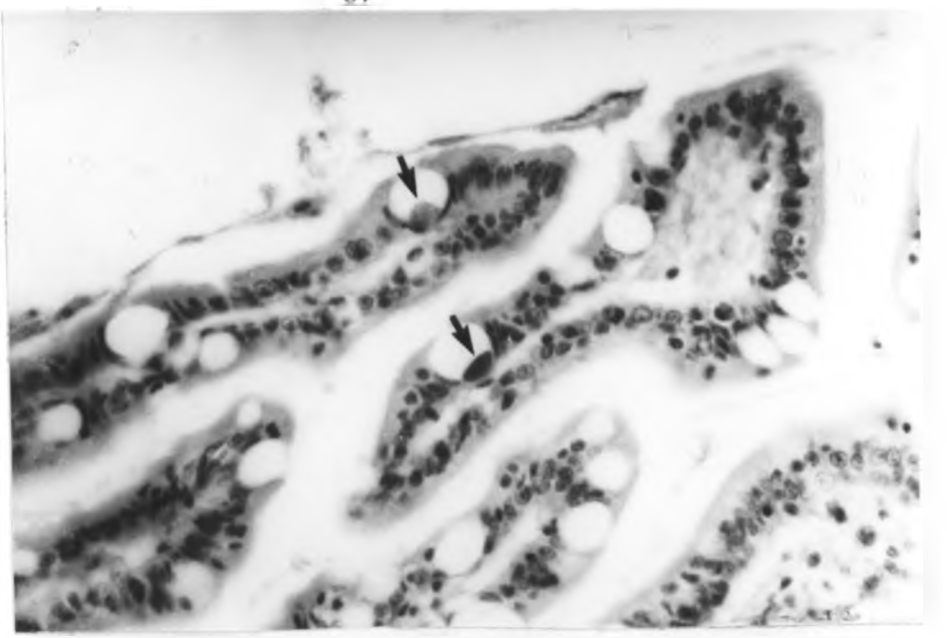


Figure 4.5: Macrogametocytes of *B. wallacei* in Goblet cells (arrows) of the ileum of a cat 16 days post-infection. H&E x400.

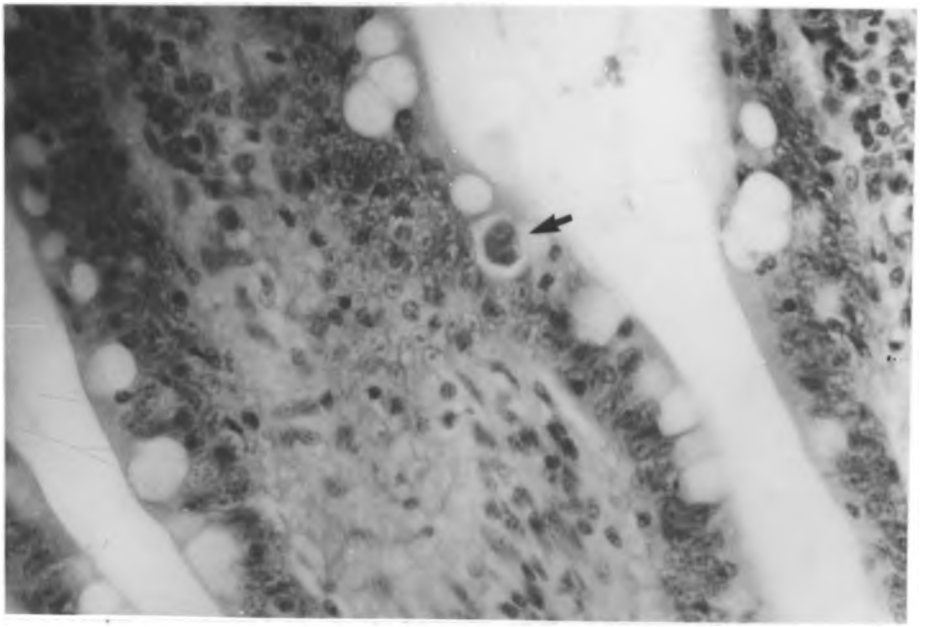


Figure 4.6: Macrogamete of *B. wallacei* in a Goblet cell (arrow) of the ileum of a cat 16 days post-infection. H&E x630.

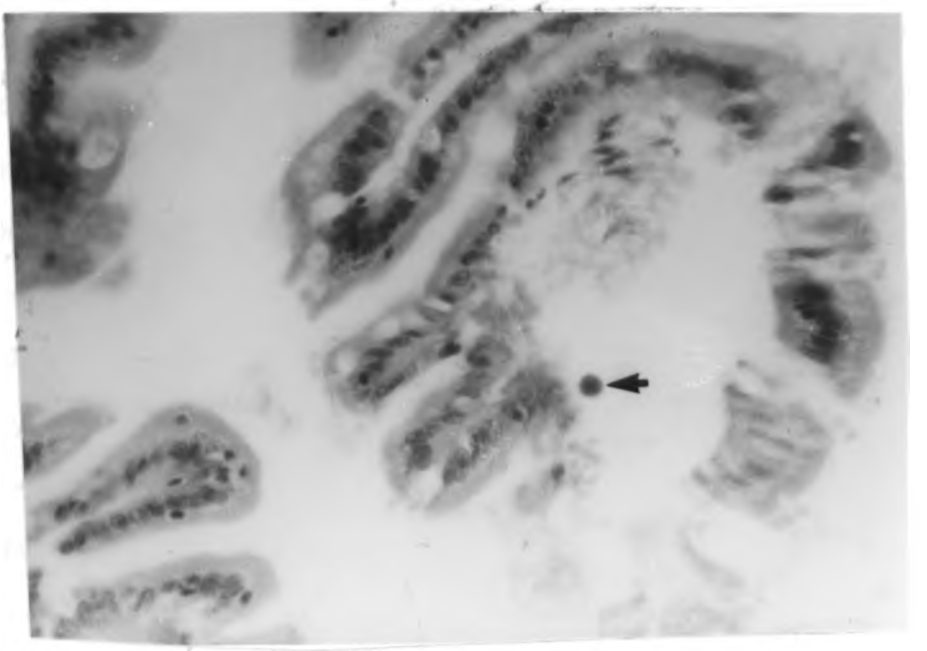


Figure 4.7: An extruded oocyst of *B. wallacei* (Arrow) in the ileal lumen of a cat 16 days post-infection. H&E x400.

4.5 DISCUSSION

The endogenous stages of the five genera of coccidia of cats mostly occur in the small intestines. Dubey and Frenkel, (1972) described the development of *Toxoplasma* where five different asexual stages(type A - E) occurred before gametogeny began. Type A measured 4 - 5 by 2 - 3 μm, type B, 3 - 17 by 2 - 11μm, type C, 9 - 17 by 9 - 11 μm, type D, 4 - 8 by 3 - 8 μm and type E, 4 - 5 by 3 - 8 μm. Macrogametocytes were 7 - 8 by 4 - 7 μm, microgametocytes were 7 - 10 by 5 - 8 μm and the microgametes 4 - 5 μm.

Hammondia develops much like *Toxoplasma*. The asexual stages resemble type D and E of *Toxoplasma* (Frenkel and Dubey, 1975).

Bradyzoites of *Sarcocystis* species develop directly into gametes (without schizogony) and oocysts sporulate in the lamina propria (Dubey, 1976, 1977; Ruiz and Frenkel, 1976). Microgametocytes of *S.muris* measure 6.5 μm (Ruiz and Frenkel, 1976).

Shah (1971) described in details the stages of *Isospora felis*. Three generation schizonts occurred before gametogony was initiated. The first generation schizonts measured 11 - 30 by 10 - 23 μm and contained merozoites 11 - 15 by 3 - 5 μm. second and third generation schizonts developed within the same host cell. The third generation schizonts measured 12 - 16 by 4 - 5 μm and contained merozoites 6 - 8 by 1 - 2 μm. The mature macrogametocytes were 16 - 22 by

8 - 13 μm and microgametocytes were 24 - 72 by 18 - 32 μm. The entire cycle occurred in epithelial cells and always above the host cell nuclei.

In the present study, asexual and sexual stages of *Besnoitia wallacei* were observed. The asexual stages occurred in the intestinal mucosa and the liver, between day 6 and 16 when the last cat was killed. While the sexual stages occurred in the intestinal mucosa between day 10 and 16 post-infection. Ito *et al* (1978) reported on endogenous stages of *B. wallacei* between day 6 and 19 post infection,but they did not observe microschizonts in epithelial cells and macroschizonts in the liver. In the present study microschizonts occurred in epithelial cells and macroschizonts in the lamina propria of small intestines and in the liver. These stages have been identified in similar locations by Wallace and Frenkel (1975) and Frenkel (1977).

Microschizonts in epithelial cells measured 22.6 by 14.7 μm (15.4 - 30 by 10.6 - 24 μm). These were larger than the *Toxoplasma* types described by Dubey and Frenkel (1972) and those of *Hammondia* described by Frenkel and Dubey (1975). The sizes were similar to those of *Isospora felis* described by Shah (1971).

Macroschizonts in the liver and lamina propria of small intestines measured 53 - 90 by 33.6 - 77.6 μm. These were in the same range as those described by Ito *et al* (1978), but smaller than those reported by Frenkel, (1979) which were

500-800 μm in diameter. The schizonts in the lamina propria were closely associated with blood vessels and the parasitized cells could be endothelial or intimal cells as suggested by Frenkel (1977). The development of schizonts in both epithelial cells and the lamina propria was concurrent. There were no indications of different generations. It therefore appears likely that *Besnoitia wallacei* has only one generation schizogony.

Sexual stages were seen between day 10 and 16 of infection and included macrogametocytes, macrogametes and microgametocytes. The macrogametocytes measured 11.4 by 8.3 μm. Mature macrogamete measured 12.9 by 8.8 μm and the microgametocytes measured 10.9 by 8.44 μm. These stages were similar in size to those reported by Wallace and Frenkel (1975) and Frenkel (1977). They were smaller than those of *Isospora felis* reported by Shah (1971), but larger than those reported on *Toxoplasma* (Dubey and Frenkel, (1972), *Hammondia* (Frenkel and Dubey, 1975) and *Sarcocystis muris* (Ruiz and Frenkel, 1976).

Although Ito *et al* (1978) did not report on schizonts in the liver, my findings were much like theirs, both in time of observation of endogenous stages and size of macroschizonts.

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CHAPTER FIVE

5 INVESTIGATION OF THE CAT AS THE FINAL HOST FOR THE BESNOITIA SPECIES AFFECTING GOATS IN KENYA AND THE ROLE OF MICE AND RATS

5.1 SUMMARY

An investigation was conducted to ascertain whether the domestic cat is the final host for the *Besnoitia* species affecting goats in Kenya. A total of 28 cats were used. Goat tissues heavily infected with *Besnoitia* cysts and freed bradyzoites were orally fed to cats. Faecal samples were examined for *Besnoitia* oocysts. *Besnoitia* oocysts were not recovered. The role played by mice and rats in the cyclic transmission of the species was also investigated by injecting them with bradyzoites. Neither *Besnoitia* cysts were observed at post-mortem, nor were their tissues infectious to cats.

5.2 INTRODUCTION

Caprine besnoitiosis has been in existence in Kenya as early as 1955, as demonstrated in dried skin sections (Bwangamoi,1968). The first indications of a clinical disease were the observations of *Besnoitia* cysts in the eyelids of goats (Heydorn *et al.*, 1984). This was followed by an outbreak in Marimanti and Kiburine sheep and goat projects in Meru district (Bwangamoi *et al.*, 1989). Since then, the disease has

been reported in Embu, Isiolo, Nakuru, Kitui, Turkana (Bwangamoi, 1989), Kwale and Taita-Taveta districts.

The life-cycle of *Besnoitia* species is still obscure. However, cats can transmit *Besnoitia besnoiti*, *B. darlingi* and *B. wallacei* through oocysts in faeces (Peteshev, *et al.*, 1974; Wallace and Frenkel, 1975; Smith and Frenkel, 1977, 1984). Although the species affecting goats in Kenya has not been classified, it is thought to be a strain of *Besnoitia besnoiti* (Bwangamoi, 1967). It was therefore suspected that the cat was the final host and its role in transmission was investigated.

5.3 MATERIALS AND METHODS

5.3.1 SOURCE OF INFECTED GOAT TISSUES.

Following an outbreak of caprine besnoitiosis at the Bachuma sheep and goat project (Coast province), seven goats with numerous scleral conjunctival cysts were selected and transported to the Department of Veterinary Pathology and Microbiology, University of Nairobi.

Biopsies were taken from ear tips, processed for microscopic examination and revealed numerous *Besnoitia* cysts (Fig. 5.1). The goats were euthanized by intravenous injection of pentobarbitone sodium and examined for *Besnoitia* cysts in their tissues. Pieces of the skin, tendons, fascia and muscles from the lower limbs which were heavily infected with grossly visible cysts were fed to cats under investigation. Pieces of

fascia with grossly visible cysts were carefully removed from the carcasses for bradyzoite recovery, (Section 5.3.3)

5.3.2 INFECTION OF CATS.

A group consisting of seven adult cats and two kittens, 2-3 months old was used. Six of the adults and the two kittens were fed heavily infected tissues from goats. One adult cat was not fed infected tissues and was used as a control. Their faecal samples were examined daily for *Besnoitia* oocysts for 30 days post-infection. All cats were killed on day 30 post-infection. Small tissue were taken from the intestines, liver, kidney, lungs, and brain and preserved in 10% formalin for histological examination.

5.3.3 RECOVERY OF BRADYZOITES FROM INFECTED GOAT TISSUES.

Pieces of fascia, 1-2 cm long with grossly visible cysts were carefully removed from the infected goat carcasses. They were collected in sterile universal bottles containing 400,000 I.U. penicillin and 500 mg streptomycin (PEN & STREP^{R}, Norbrook laboratories Ltd Newry). Universal bottles were kept immersed in ice cubes to reduce bacterial contamination.

To release bradyzoites from cysts, the pieces of fascia were ground using a sterile mortar and pestle. Phosphate buffered saline, (10 mls) (PBS, pH 7.8) was added to prevent death by desiccation.

The ground fascia was then transferred into a sterile cheese cloth carefully fitted into a funnel. Bradyzoites were washed into sterile flat bottomed flasks (100ml) by pouring PBS into the funnel and carefully stirring with a glass rod.

The filtrate was concentrated by centrifugation at 1,000 r.p.m. for 10 minutes. The viability of the bradyzoites was confirmed by observing the gliding motility under a light microscope. The number of bradyzoites was estimated using a haemocytometer and diluted to 1 x 10^6 organisms per ml using PBS. These were used for inoculation of cats, mice and rats (Section 6.3.4 and 6.3.5).

5.3.4 INFECTION OF CATS WITH BRADYZOITES

Two adult cats were orally infected with approximately 1 x 10^6 bradyzoites recovered from infected goat tissues (Section 5.3.3). Faecal samples were examined for *Besnoitia* oocysts for 30 days post-infection.

5.3.5 MICE AND RATS INOCULATION

5.3.5.1 RATS

Rats were divided into two groups of ten members each. One group was injected subcutaneously with approximately 1 x 10^6 bradyzoites each. While the other group received a similar dose intraperitoneally. All rats were killed on day 60 of infection and carcasses examined for *Besnoitia* cysts in the skin and visceral organs. Small samples were taken from the

intestines, skin, heart, liver, kidneys and lungs fixed in 10% formalin and processed for histological examination. The rest of the carcasses were fed to cats (Section 5.3.6).

6.3.5.2 **MICE**

The 20 mice were divided and treated as rats above

5.3.6 **INFECTION OF CATS WITH MICE AND RAT TISSUES**

Four adult cats were used, the first cat was fed on carcasses of rats infected subcutaneously (Section 5.3.5). The second, third and fourth cats were fed carcasses of rats infected intraperitoneally and mice infected both ways in that order. Faecal samples were examined for *Besnoitia* oocysts for 30 days post-infection.

5.3.7 **STRESS AND INFECTION OF CATS**

Heavy helminth infection or the administration of glucocorticoids above the physiological levels are stressful to animals. This predisposes the animals to secondary pathogen infections. Twelve adult cats under natural or artificial stress were tested.

5.3.7.1 **NATURAL STRESS**

Six adult cats naturally infected with helminths were used. They had a faecal strongyle egg count of over 1,000 and over 100 ascarid eggs per gram of faeces. They were fed on

Besnoitia infected tissues from goats (Section 5.3.1) and killed on day 30 post-infection for post-mortem.

5.3.7.2 ARTIFICIAL STRESS

Seven adult cats were injected intramuscularly with dexamethasone (Dexazone^{R},Bimeda, Dublin) at a dosage rate of 0.04 mg/kg. This was done daily for 5 days prior to infection and continued for 7 days post-infection.

Of the seven cats, two were fed on tissues from goats with heavy *Besnoitia* infection (Section 5.3.1), two were orally infected with approximately 1 x 10^{6} bradyzoites from goats (Section 5.3.3), two were fed on rat carcasses previously infected with bradyzoites (Section 5.3.5) and one was not infected and was used as a control. Faecal samples were collected from all cats and examined for *Besnoitia* oocysts.

5.4 RESULTS

5.4.1 INFECTION OF CATS

None of the 28 cats used in the experiments shed *Besnoitia* oocysts in their faeces. The six cats naturally infected with helminths produced strongyle and ascarid eggs in their faeces (Section 5.3.7.1)

At post-mortem, *Ancylostoma braziliense*, *Toxocara cati* and tapeworms were seen in the small intestines of the six cats naturally infected with helminths (Section 5.3.7.1). At

histology the lung tissue had numerous sections of lungworms (Fig. 5.2).

5.4.2 MICE AND RAT INOCULATION

At post-mortem there were no gross or histological lesions to indicate *Besnoitia* infection in mice and rats in all organs examined.

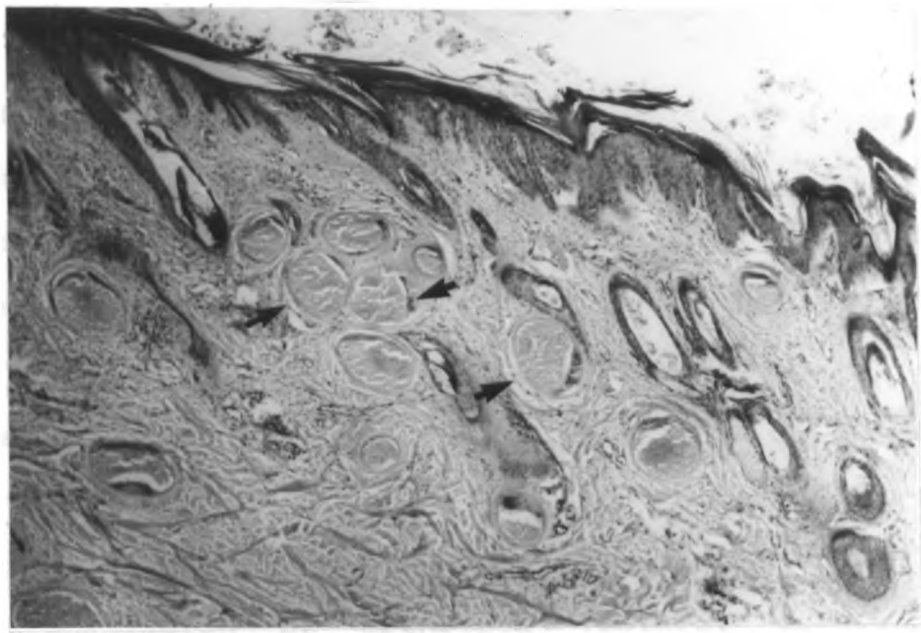

Figure 5.1: *Besnoitia* cysts (arrows) in the skin section of a naturally infected goat used to infect cats. H&E x40.

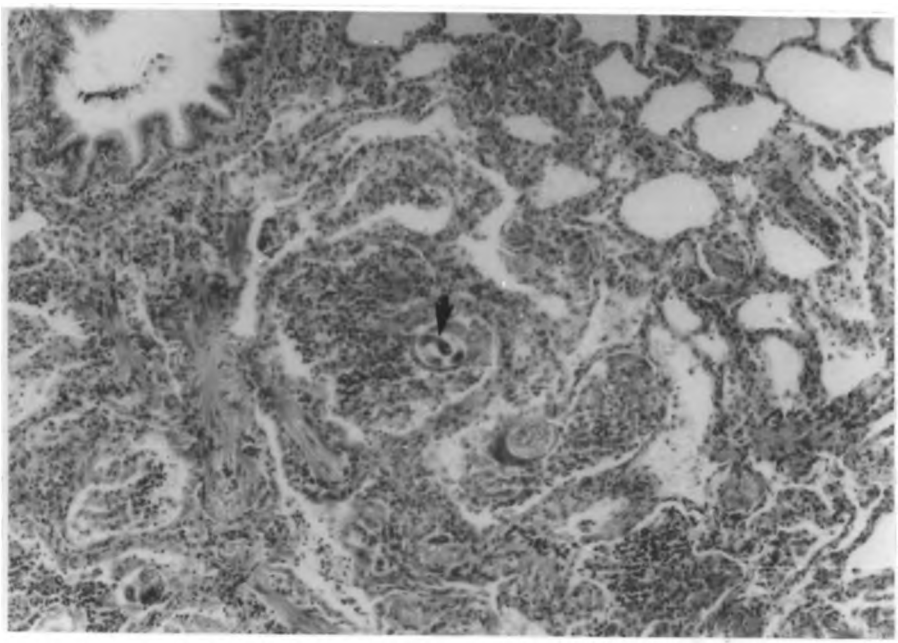

Figure 5.2: Lung tissue from a naturally infected cat used in the investigation of *Besnoitia* transmission showing a cross-section of a lung worm (arrow). H&E x100.

5.5 DISCUSSION

The life-cycle of member of the genus *Besnoitia* is still obscure. Several methods of transmission have been suggested and investigated. Hofmeyer (1945) suggested the possibility of transmission of *Besnoitia besnoiti* by contaminative methods. This was investigated by Bigalke (1968) where infected animal cohabited with healthy animals. The healthy animals became infected. Bigalke (1968) also investigated the possibility of mechanical transmission by arthropod vectors. *Glossina brevipalpis*, *Stomoxys calcitrans* and Tabanids were found to be effective vectors.

Cyclic transmission by cats has been reported for *Besnoitia besnoiti* (Peteshev, *et al.*, 1974), *B. darlingi* (Smith and Frenkel, 1977, 1984) and *B.wallacei* (Wallace and Frenkel, 1975; Ito *et al.*, 1978; McKenna and Charleston, 1980b). In the present investigation, cats could not be infected with the *Besnoitia* species affecting goats in Kenya directly from goats or through mice and rats. Similarly, attempts to transmit to cats, *Besnoitia besnoiti* from infected cattle (Rommel, 1975, Diesing *et al.*, 1988), *Besnoitia jellsoni* (Wallace and Frenkel, 1975) and *Besnoitia tarandi* from infected caribou (Glover *et al.*,1990) were unsuccessful. It is therefore possible that *Besnoitia* as a genus utilizes different final hosts for different species. Such an observation has been made for *Sarcocystis* which belongs to the

same family (*Sarcocystidae*, Poche, 1913) as *Besnoitia* and utilizes the cat, man and dogs as final hosts (Heydorn *et al.*,1975). Further investigations are thus necessary to establish the final hosts for all *Besnoitia* species.

Although *Besnoitia* species have a wide range of natural and experimental intermediate hosts, the species affecting goats in Kenya could not be transmitted to mice and rats as demonstrated by lack of tissue cysts. Similarly, Bwangamoi *et al* (1989) did not observe scleral conjunctival cysts in cattle grazing alongside infected goats. Also Pak (1976) and Kaggwa *et al* (1979) could not demonstrate cysts in mice inoculated with *Besnoitia besnoti* from cattle. Wallace and Frenkel (1975) and McKenna and Charleston (1980b) could not infect guinea pigs with *Besnitia wallacei* oocyst from cat faeces. Therefore, there is a limit to intermediate hosts that can be infected by specific *Besnoitia* species. Further investigations on the intermediate host range for *Besnoitia* species is necessary for effective control measures to be taken.

CHAPTER SIX

6 INFECTION OF GOATS WITH BESNOITIA WALLACEI OOCYSTS FROM CAT FAECES

6.1 SUMMARY

Besnoitia wallacei oocysts were orally inoculated to 5 goats. None of the five goats developed clinical besnoitiosis. Tissues from these goats were fed to three cats. Two of the three cats produced *Besnoitia* oocysts within 12 days of infection and the third cat which died on day 10 post infection had *Besnoitia* endogenous stages in the small intestines.

6.2 INTRODUCTION

Besnoitia species are capable of parasitizing a wide range of natural and experimental intermediate hosts. Transmission can be from one intermediate host to another through bradyzoites or tachyzoites inoculation (Schneider, 1967a; Bigalke, 1968; Glover, *et al.*, 1990) or from the final host to intermediate hosts through oocyst ingestion (Peteshev, *et al.*, 1974; Wallace and Frenkel, 1975; Smith and Frenkel, 1977).

Oocysts of *Besnoitia wallacei* are infectious to rats, mice and rabbits (Wallace and Frenkel, 1975; McKenna and Charleston, 1980b). The susceptibility of domestic livestock

to this species cannot be ruled out and needs to be investigated. This work was therefore meant to ascertain whether this species is infectious to goats through oocyst ingestion.

6.3 MATERIALS AND METHODS

6.3.1 SOURCE OF CLEAN GOATS

Seven, 9-12 months old goats of mixed sexes were obtained from a locality in Kajiado District of Kenya where besnoitiosis had not been reported. The goats were clinically examined for any infection and for besnoitiosis by the method of Bigalke and Naude, (1962) at the time of purchase and passed as healthy. They were transported to the Animal Compound of the Department of Veterinary Pathology and Microbiology, University of Nairobi, where they were fed on hay, concentrates, minerals and water provided *ad libitum*.

The goats were randomly assigned to two groups and housed separately in fly proof pens. Group one consisted of five goats used as the experimental (Infected) group while group two consisted of two goats used as the control group. Ear tags were put for easy identification. Group one members were identified as G31, G32, G33, G35 and G37 and group two as G34 and G36. The goats were allowed 28 days to adopt to housing. They were maintained on hay, concentrates, mineral salts and water provided *ad libitum*.

During the adaptation period faecal samples were taken weekly and examined for the presence of helminths and coccidia. On the second week, all goats were dewormed using 10% albendazole (Valbazen^{R}, Kenya Swiss chemical company) and orally treated with furazolidone (Fuzol^{R}, Cosmos Ltd) at a dosage rate of 20 mg/kg for the control of coccidial infection.

6.3.2 SOURCE AND ADMINISTRATION OF INFECTIOUS MATERIAL

Besnoitia wallacei oocysts recovered from cats previously infected with mice and rat tissues were used (Chapter 3, section 3.3.6.2). The inoculum was estimated by a haemocytometer. Goats in group one were orally infected with approximately 10^{6} sporulated oocysts each.

6.3.3 CLINICAL EXAMINATION

Rectal temperature was taken every morning for 28 days before infection and for 30 days post-infection. Blood samples (2ml) were collected from the jugular vein twice a week before and after infection using a 19 gauge needle (1.5" long) and a 5 ml syringe. The samples were put in Bijou bottles containing sodium-ethylene-diamine-tetra-acetate (EDTA) prior to laboratory analysis.

Erythrocyte counts, total leucocyte counts, and the mean corpuscular volume were determined by a coulter counter (mode Z M,coulter Electronics Inc. Florida). The haemoglobin

concentration was estimated by a haemoglobinometer (Coultronics, France. S.A Margency). Total plasma proteins were estimated by a refractometer.

For Micro-haematocrit determination, blood samples were drawn into heparinized capillary tubes, centrifuged for 5 minutes and read off on a micro-haematocrit reader (Hawskey and Sons Ltd., London). Buffy coat smears were made, stained for 15 - 20 minutes in Giemsa solution diluted to 1:5 and examined for blood parasites.

For differential leucocyte counts thin blood smears were made, stained in Giemsa solution diluted to 1:5 for 15 - 20 minutes. One hundred leucocytes were differentiated. The smears were also examined for blood parasites. The standard ratios (Mean corpuscular volume and Mean corpuscular haemoglobin concentration) were calculated using the formulae provided by Archer and Jeffcott (1977).

For 78 days post-infection both groups were examined daily for lymph node enlargement, scleral conjunctival cysts, skin and testicular changes characteristic of besnoitiosis.

For statistical analysis temperature and haematological data was compared pre-infection and post-infection for both experimental and control groups. Analysis of variance was then performed as described by Steel and Torrie (1980).

All goats were euthanized by intravenous injection of pentobarbitone sodium between day 78 and 80 post infection and postmortem performed as described by Jones and Gleiser (1954).

Small tissue samples were taken from the lungs, heart, liver, spleen, kidneys, lymph nodes, skeletal muscles, intestines, rumen, reticulum, omasum, abomasum testes and from the skin, fixed in 10% formalin and processed for histological examination.

6.3.4 INFECTION OF CATS

Small pieces of fresh muscles,stomachs, lungs and liver from goat G32, G33, G34, G35 and G36 (Section 6.3.3) were pooled for each goat and fed to four cats with corresponding identification. Faecal samples were collected daily for 35 days post-infection and examined for *Besnoitia* oocysts.

6.4 RESULTS

6.4.1 CLINICAL EXAMINATION

Strongyle eggs (200-1000 eggs per gram of faeces) and *Eimeria* oocysts were present in all faecal samples, but cleared after treatment. The rectal temperatures and some haematological parameters (Appendices 1-4) after analysis did not show any significant change that could be attributed to *Besnoitia* infection. However, the pre-infection erythrocyte (Rbc) and eosinophil counts and the micro-haematocrit readings (PCV) were higher than the post-infection counts among the experimental and control groups.

6.4.2 POST-MORTEM

On the intestines of G32, nodules caused by *Oesophagostomum* infection were seen. Larvae of *Taenia hydatigena* (*Cysticercus tenuicollis*) were seen attached to the peritoneal organs of G36 and G37. In the thoracic cavity, foci of pleural adhesions were seen in G33 and G36. There were no grossly visible *Besnoitia* cysts.

Microscopically, lesions were observed in sections of the lungs, liver and muscles. In the lungs of G31, G32, G33 and G37 there were foci of mononuclear cell infiltration (Fig. 6.1), mainly lymphocytes and macrophages (Fig. 6.2). Liver sections from G32, G33, G35 and G37 had foci of necrosis and cellular infiltrations (Fig. 6.3) mostly comprising of lymphocytes, macrophages and neutrophils (Fig. 6.4). In cardiac and skeletal muscles, sections of *Sarcocystis* (Fig. 6.5) were seen in all infected and control goats.

6.4.3 INFECTION OF CATS

Two of the three cats fed tissues from experimental goats produced *Besnoitia* oocysts on day 12 of infection. Microscopically, intestinal sections from the third cat that died on day 10 post-infection revealed *Besnoitia* endogenous stages morphologically similar to those described in Chapter 4. None of the cats fed on tissues from the control group of goats produced *Besnoitia* oocysts.

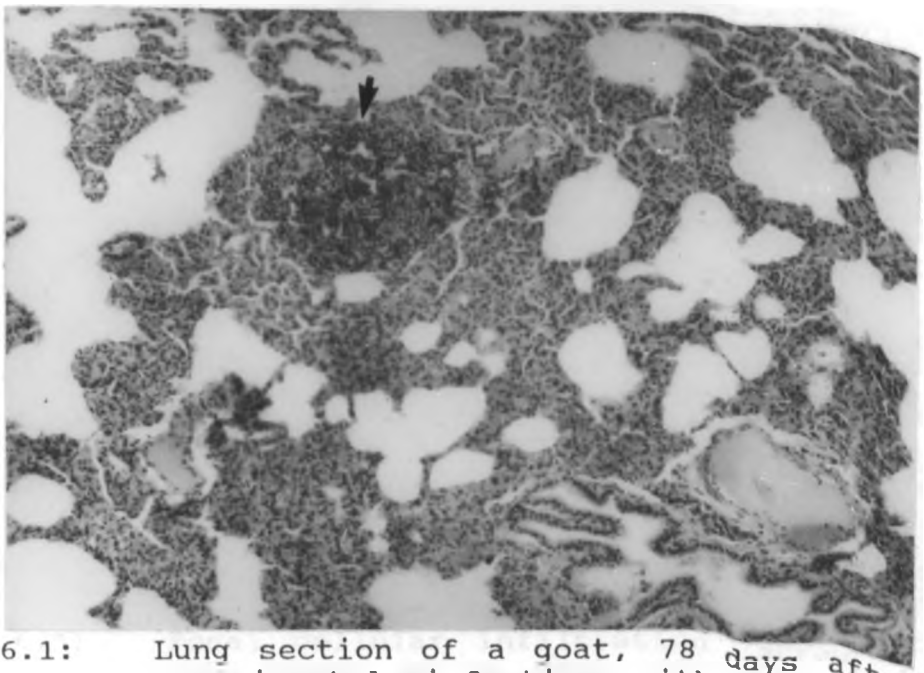

Figure 6.1: Lung section of a goat, 78 days after experimental infection with *Besnoitia wallacei* oocysts, showing focal cellular infiltrations (arrows). H&E x100.

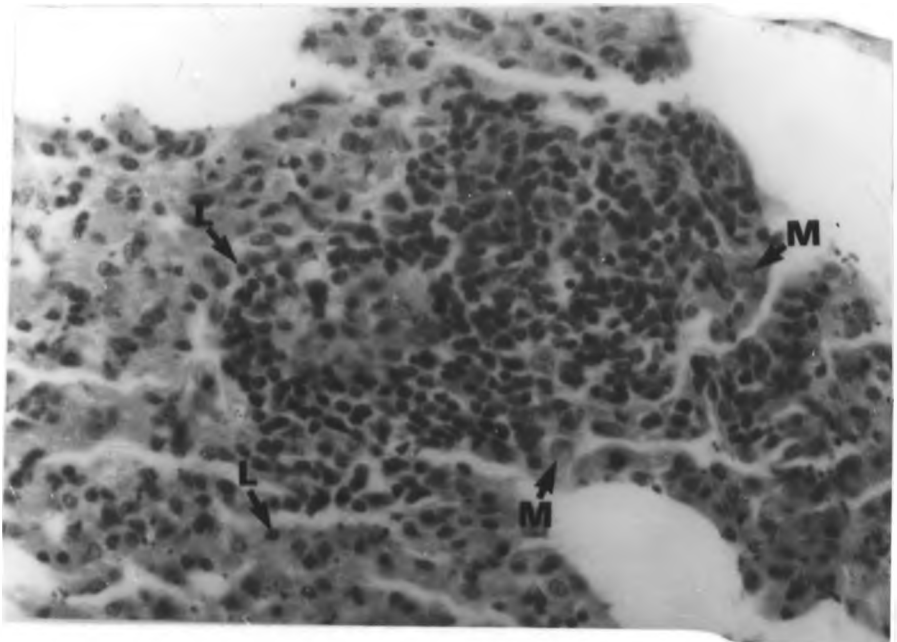

Figure 6.2: Lung section of a goat 78 days after experimental infection with *Besnoitia wallacei* oocysts, showing mononuclear cells infiltration, mainly macrophages (M) and lymphocytes (L). H&E x630.

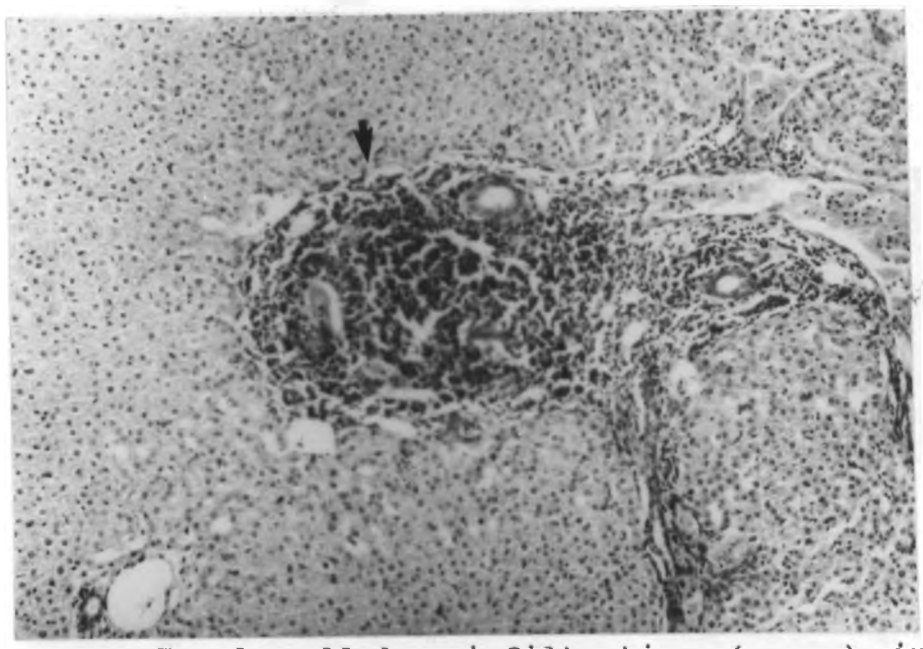

Figure 6.3: Focal cellular infiltration (arrow) in the liver of a goat 78 days after experimental infection with *Besnoitia wallacei* oocysts. H&E x100.

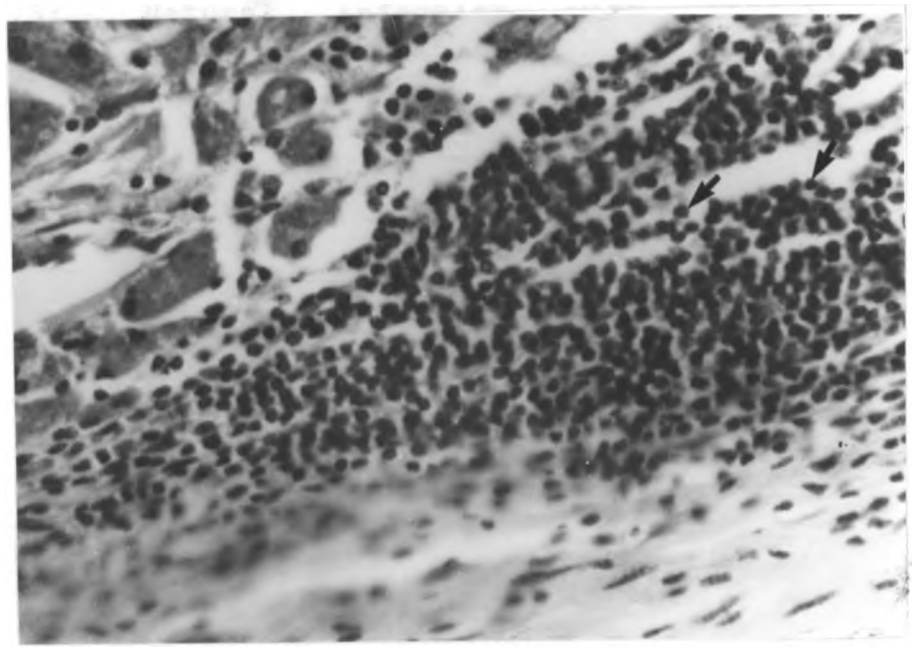

Figure 6.4: A section of the liver of a goat 78 days after experimental infection with *Besnoitia wallacei* oocysts, showing lymphocytic infiltration (arrows). H&E x400.

Figure 6.5: Natural infection with *Sarcocystis* (Arrow) in the cardiac muscle of a goat used in the experimental infection with *Besnoitia wallacei*. H&E x100.

6.5 DISCUSSION

Oral inoculation of goats with oocysts of *Besnoitia wallacei* resulted in a subclinical infection. The high pre-infection erythrocyte counts and micro-haematocrit reading was not related to *Besnoitia* infection, but may be explained by the fact that the goats were initially not used to being handled and that their age was increasing (Archer and Jeffcott, 1977). The high pre-infection eosinophil counts could have been a result of helminth and coccidia infection which cleared after treatment.

Microscopically, the lungs and the liver had focal areas of mononuclear cell infiltration in addition to focal areas of necrosis seen in the liver. Although no parasites were demonstrated in this foci, the lesions were similar to those observed in cites of parasite development in cattle infected with *Besnoitia besnoiti* (Basson *et al* 1970). However, the parasites in these foci may have been destroyed by the host's defence mechanism by the goats were euthanized.

The production of *Besnoitia wallacei* oocyst by two of the three cats fed on tissues of the experimentally infected goats and the demonstration of endogenous stages in the third cat was an indication that the goats got infected. Similar observations were made for other tissue cyst-forming coccidian parasite. Ito *et al* (1978) could not detect tissue cysts in hamsters infected with *Besnoitia wallacei*, but cats fed on organs from these animals resulted in oocysts production.

Dubey (1975) and Shimura and Ito (1987) could not demonstrate tissue cysts in dogs and goats infected with *Hammondia hammondi*, but cats fed on their tissues produced oocysts. Failure to demonstrate *Besnoitia* developmental stages in goat tissues can be attributed to a poor infection of the host by the parasite and the small proportion of tissues examined at histology in relation to the entire goat size.

CONCLUSIONS

From the above experiments the following conclusions were made.

1. That *Isospora felis*, *I. rivolta*, *Sarcocystis muris*, *Toxoplasma gondii* and *Besnoitia wallacei* infect cats around Kabete.
2. That the development of *Besnoitia wallacei* in the cat involves asexual stages with one generation schizogony in the intestines and the liver and sexual stages in the intestines.
3. That the domestic cat, mice and rats may not be involved in the cyclic transmission of the *Besnoitia* species affecting goats in Kenya.
4. That the goat is a poor intermediate host of *Besnoitia wallacei*.

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APPENDICES

APPENDIX 1

Weekly temperature means for experimental and control groups, pre- and post-goat inoculation with *Besnoitia wallacei* oocysts.

Time in Weeks	Temperature in °C	
	Experimental group	Control group
1	38.6	38.4
2	38.6	38.1
3	38.3	38.0
4	38.0	38.1
5	38.1	37.9
6	38.4	38.1
7	38.4	38.1
8	38.4	38.2
9	38.5	38.2

KEY

WEEK 1-4: Period before inoculation
WEEK 5-9: Period after inoculation

NOTE: There was no significant change in temperature and the data collection was therefore stopped on the ninth week.

APPENDIX 2

Mean weekly erythrocyte counts and erythrocyte indices for experimental and control groups, pre- and post-goat inoculation with *B. wallacei* oocysts

Time in Weeks	RBC ($X10^6$/ml)		PCV (%)		MCV (fl)		MCHC (gm%)	
	EXP.	CONT.	EXP.	CONT.	EXP.	CONT.	EXP.	CONT
1	16.7	17.8	29.2	29.8	37.2	36.8	33	31
2	14.4	17.3	29.1	29.8	38.2	36.8	33	33
3	13.3	15.2	26.8	25.8	37.7	38.0	36	34
4	15.0	14.4	27.8	25.8	38.3	39.3	33	33
5	13.7	11.7	27.4	24.5	39.8	37.5	32	31
6	15.0	13.1	27.0	25.8	37.3	38.5	34	35
7	13.8	13.2	27.1	26.3	36.6	36.3	36	36
8	13.0	12.1	27.8	24.3	37.3	36.8	35	33
9	12.6	12.5	24.7	22.5	37.6	37.3	33	32
10	13.5	12.6	26.0	23.3	35.9	37.5	34	34
11	12.3	12.3	25.4	23.3	36.4	36.8	34	36
12	13.0	13.1	25.2	24.8	37.1	36.5	38	37
13	11.9	12.7	25.5	24.0	38.0	37.8	36	36
14	12.5	12.5	25.5	23.8	39.0	37.8	41	39
15	12.8	12.3	25.7	23.3	37.8	37.5	35	35

KEY

RBC - Total erythrocyte counts
PCV - Packed cell volume
MCV - Mean Corpuscular volume
MCHC - Mean corpuscular haemoglobin concentration
EXP - Experimental group
CONT - Control group
WEEK 1-4 Period before infection
WEEK 5-15 Period after infection

APPENDIX 3

Mean weekly leucocyte counts for experimental and control groups, pre- and post-goat inoculations with *B. wallacei* oocysts.

Time in Weeks	WBC ($x10^3$)/ml		N ($x10^3$)/ml		L ($x10^3$)/ml		E ($x10^3$)/ml	
	EXP.	CONT.	EXP.	CONT.	EXP.	CONT.	EXP.	CONT.
1	17.7	20.4	4.8	4.6	12.3	15.3	0.6	0.5
2	17.0	21.7	4.4	6.7	12.0	14.6	0.6	0.4
3	17.2	16.6	4.2	4.2	12.7	12.2	0.3	0.3
4	16.6	15.4	3.8	4.3	12.6	10.8	0.2	0.3
5	18.5	17.3	4.0	4.0	14.2	13.1	0.3	0.2
6	19.1	16.1	5.4	3.6	13.5	12.4	0.2	0.1
7	18.4	18.4	4.7	4.4	13.5	13.9	0.2	0.1
8	19.2	17.8	5.6	5.2	13.5	12.4	0.1	0.2
9	15.6	13.4	3.9	3.3	11.5	9.8	0.2	0.3
10	18.4	15.4	4.8	4.8	13.5	10.4	0.1	0.2
11	16.3	14.7	4.4	3.5	11.7	9.9	0.2	0.3
12	15.7	14.3	4.2	4.3	11.3	9.8	0.2	0.2
13	16.5	13.7	4.2	4.0	12.0	9.4	0.3	0.3
14	16.7	14.8	4.1	4.2	12.6	10.5	0.2	0.1
15	17.9	17.8	5.3	5.6	12.5	12.0	0.1	0.2

KEY

WBC - Total leucocyte counts
N - Total Neutrophil counts
L - Total Lymphocyte counts
E - Total Eosinophil counts
EXP. - Experimental groups
CONT. - Control groups
WEEK 1-4 Period before infection
WEEK 5-15 Period after infection

APPENDIX 4

Mean weekly total plasma proteins and haemoglobin concentrations for experimental and control groups, pre- and post-goat inoculation with *B. wallacei* oocysts.

Time in weeks	Total Protein (gm%)		Haemoglobin Concentration (gm%)	
	EXP.	CONT.	EXP.	CONT.
1	7.7	8.1	9.7	9.1
2	7.6	7.5	9.6	10.0
3	7.5	7.1	9.5	8.8
4	7.7	7.6	9.0	8.6
5	7.7	6.9	8.6	7.6
6	7.8	6.9	9.4	8.9
7	8.1	7.1	9.9	9.6
8	7.6	7.3	8.9	7.9
9	6.9	6.3	8.2	7.1
10	7.5	6.7	8.7	7.8
11	7.2	6.7	8.8	8.9
12	7.7	6.8	9.5	9.3
13	7.7	6.9	9.2	8.5
14	7.8	7.2	10.3	9.2
15	7.6	7.3	8.8	8.1

KEY

EXP. - Experimental group
CONT. - Control group
WEEK 1-4 - Period before inoculation
WEEK 5-15 - Period after inoculation