

**U A COMPARATIVE CLINICAL AND PATHOLOGICAL  
STUDY OF BESNOITIA STRAINS INFECTIVE TO CATTLE  
AND GOATS U**

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**A thesis submitted in partial fulfillment for the Degree of**

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## DECLARATION

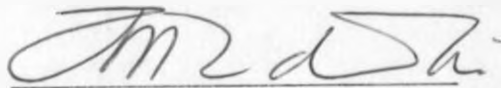
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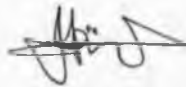
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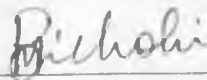
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**DEDICATION**

To My Dear Parents, Richard and Millicent.

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## ABSTRACT

*Besnoitia* are cyst-forming coccidian protozoa causing besnoitiosis in domestic and wildlife hosts worldwide. Economic losses result from reduced weight gain and milk yield, meat of poor quality and skin unsuitable for tanning, long convalescence period and predisposition to secondary infections. *Besnoitia* organisms are morphologically identical and closely related antigenically but specific *Besnoitia* species have been identified in cattle, horses, antelopes and rodents. Outbreaks of caprine besnoitiosis have been reported in Kenya but the identity of *Besnoitia* parasites found naturally in goats has not been clarified. It has been assumed previously that *Besnoitia besnoiti* causes natural besnoitiosis in both cattle and goats. In this study, the relationship between artificial infections of *Besnoitia besnoiti* of cattle and *Besnoitia* sp. of goats was investigated.

Two groups of local goats and New Zealand white rabbits were infected with either of the two parasites isolated from chronic natural cases of besnoitiosis in the same geographical region. The clinical signs, haematological and pathological aspects of the disease were studied.

The clinical signs demonstrated biological differences in virulence between the two parasites in the experimental hosts. Bovine *Besnoitia besnoiti* produced an acute and fatal anasarca syndrome in goats and rabbits while caprine *Besnoitia* sp. elicited a transient febrile reaction in goats and rabbits, and a chronic form only in goats. While rabbits died within 18 hours following infection with bradyzoites of bovine *Besnoitia besnoiti*, they were refractory to the bradyzoites of caprine *Besnoitia* sp. Haematological and histopathological findings revealed different degrees of cellular response but similar patterns of parasite distribution between the two *Besnoitia* sp. Total leucocyte cell counts in goats infected with either

isolate produced diphasic leucocytic response curves coinciding with the initial pyrexia and the acute clinical phases respectively. Viscerotropic *Besnoitia* cysts were demonstrated in various organs of animals infected with bovine *B. besnoiti* and caprine *Besnoitia* sp. Caprine *Besnoitia* sp. elicited peculiar "Besnoitia granulomas" with uniform cellular arrangements in goats.

This study appears to be the first to show that the *Besnoitia* spp. found in cattle and goats in Kenya represent distinct strains or biological races of *Besnoitia besnoiti*. The name *Besnoitia granulomae* is proposed for *Besnoitia* sp. found in goats in Kenya.

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## INTRODUCTION

Besnoitiosis is an acute, subacute or chronic disease caused by coccidian protozoan parasites of the genus *Besnoitia*. It is characterized by formation of white miliary cysts in all parasitized organs or tissues. Cysts occur in almost any tissue containing blood vessels (Bwangamoi, 1979). Clinically, it is expressed as a syndrome of pyrexia, anorexia, general malaise, enlargement of superficial lymph nodes, lacrimation, anasarca, alopecia, rhinitis, pharyngo-laryngitis, tracheitis and orchitis. Since the original report on bovine besnoitiosis by Besnoit and Robin (1912), several investigators have contributed to the present knowledge of the pathology of the disease. Predilection sites for *Besnoitia* cysts are mainly confined to the veins of the head, neck, limbs and the flanks but cysts may form in any organ. The cysts are commonly encountered in the skin, subcutis, skeletal muscles, intermuscular fascia, mucosae of the anterior respiratory tracts, synovial sheaths, periosteum and testis. In severe cases, vasculitis is primary to the manifestations of anasarca, hydrothorax, hydropericardium, ascites and the degenerative and necrotic changes, particularly in the testis and skin that precede development of cysts (Basson *et al.*, 1970). A leucocyte reaction, especially monocytosis is a further characteristic feature of the syndrome (Pols, 1960).

The seasonal occurrence of besnoitiosis is significant in diagnosis but cyst organisms may be seen in the sclero-conjunctiva and the anterior nares (Bigalke, 1968; Bigalke and Naudé, 1962). Histological examination readily shows the cyst organisms in the papillary and reticular layers of the dermis and in the subcutis. *Besnoitia* antibodies are demonstrable by various serological methods most of which are hardly specific. No effective cure has been discovered

for besnoitiosis although complete recovery was reported in gerbils treated with oxytetracycline (Shkap *et al.*, 1982). Supportive treatment with broadspectrum antibiotics and some sulphonamides is valuable (Pols, 1954a, 1954b, 1960). It would appear that elimination of chronic carriers and vectors would be a reasonable control measure but this should be viewed against the fact that the disease commonly occurs in rangelands and the mode of transmission is not completely understood. Economic implications of besnoitiosis have not been studied but it is certainly of economic importance where it occurs. It causes temporary or permanent sterility in bulls, abortions in cows and renders leather unsuitable for tanning. An infected animal is cachectic and so pitiful that it is destroyed on humane grounds (Schulz, 1960).

*Besnoitia* organisms are morphologically indistinguishable and closely related antigenically. The speciation of the genus is still based on: a) the species of experimental animals infected, b) the degree of infection produced in hamsters, c) the differences in cyst sizes, d) the pathology found in the natural host (Suggs *et al.*, 1968) and e) factors of geographical incidence. Differences in virulence in various hosts (Bigalke *et al.*, 1967; Basson *et al.*, 1970) and isoenzyme electrophoresis (Le Blancq *et al.*, 1986) have been applied to differentiate bovine and antelope strains of *Besnoitia besnoiti*. Antigenic comparisons have hardly been used.

*Besnoitia besnoiti* (Marotel, 1912) is associated with disease in bovine and is believed to affect caprine hosts with similar clinical manifestations and pathology. Rabbits are very susceptible to infection with proliferative (Pols, 1954a, 1954b, 1960) and cyst forms (Bigalke, 1960; Bigalke *et al.*, 1967) of *Besnoitia besnoiti* of cattle. They develop more acute clinical signs than those in the natural hosts: cattle, goats, blue wildebeests, impala and kudu and hence serve as good experimental models for studies on bovine *Besnoitia besnoiti* infection. However,

the chronic form in rabbits is more often asymptomatic, circulating parasites are scarce to find and cyst formation in the upper lip, nostrils or internal organs are only occasionally detected.

Although evidence of spontaneous besnoitiosis in domestic goat has been reported in Kenya (Bwangamoi, 1967; Bwangamoi *et al.*, 1989; Kaliner, 1973) and Iran (Cheema and Toofanian, 1979) little is known about caprine besnoitiosis. Clinical besnoitiosis in the domestic goat (*Capra hircus*) was only discovered in the beginning of 1989 (Bwangamoi *et al.*, 1989) and the identity of *Besnoitia* parasites found naturally in goats is yet to be clarified. Countries which have done extensive research on bovine besnoitiosis such as South Africa, Israel, and the USSR have singularly not reported besnoitiosis in the goat. In East Africa caprine besnoitiosis is only currently being studied although goats have always been kept in the region. Economic losses result from reduced weight gain and milk yield, meat of poor quality and skin which is unsuitable for tanning, long convalescence period, predisposition to pneumonia and death (Bwangamoi, 1989b). Recent observations have put to test the previous assumption that one strain of *Besnoitia* was responsible for besnoitiosis in both cattle and domestic goats. In one natural outbreak of caprine besnoitiosis (Bwangamoi *et al.*, 1989), no trace of *Besnoitia* was found in cattle and sheep that had grazed alongside the affected goats for a period of two years although this could not be completely ruled out since no serological tests were carried out on the cattle and the sheep. A flock of goats admitted to the Department of Veterinary Pathology and Microbiology, University of Nairobi, expressed atypical besnoitiosis, thus enhancing speculation on the existence of a species of *Besnoitia* in goats different from *Besnoitia besnoiti* (Marotel, 1912), the cause of bovine besnoitiosis (Besnoit and Robin, 1912). In view of the continued occurrence of outbreaks of caprine besnoitiosis, it was found necessary to carry out studies on



the bovine and caprine strains of *Besnoitia*. The main objectives of this study were:

#### LITERATURE REVIEW

1. To determine whether the bovine and caprine strains of *Besnoitia* occurring in Kenya are two different organisms.
2. To study the clinical manifestations, gross lesions and histopathological changes in the acute and chronic disease in both goats and rabbits after infection with either caprine or bovine *Besnoitia* sp.

## LITERATURE REVIEW

### 2.1. AETIOLOGY

*Besnoitia* are coccidian protozoan parasites with different species causing besnoitiosis in both domestic and wildlife hosts. Frenkel (1977b) classified *Besnoitia* together with other isosporan coccidia of the genera *Toxoplasma*, *Hammondia* and *Cystoisospora* in the subfamily *Toxoplasmatinae*, family *Sarcocystidae*. The coccidia in this family produce oocysts which contain two sporocysts, and each sporocyst contains four sporozoites. This distinguishes them from eimerian coccidia, except *Isospora*, in which the oocyst contains four sporocysts each of which contains four sporozoites. Levine (1977) grouped the same parasites together and included other genera, *sarcocystis* and *Frenkelia* into one family Eimeriidae. He suggested that *Hammondia* be considered as a species of the genus *Toxoplasma*. Frenkel created the genus *Cystoisospora* for the species in the genus *Isospora* that form cysts in the intermediate hosts namely, *Isospora felis* and *Isospora rivolta*.

The morphology of the developmental stages of *Besnoitia* parasites was studied by Pols (1960) in artificially infected rabbits. *Besnoitia* reproduces by longitudinal binary fission and is morphologically indistinguishable from *Toxoplasma* (Goldman *et al.*, 1957). *Besnoitia* differs from *Toxoplasma* in the type of cysts it produces in chronic infections, in details of pathology in laboratory animals and in its serology. *Besnoitia* cysts are longer than those of *Toxoplasma* and also have a thicker wall lined by large nuclei. They are basically intracellular parasites although extracellular proliferative forms are found in the bloodstream and various organs during the early stages of the infection. Circulating forms in the blood, lung and testis smears of rabbits

vary in size from 5-9  $\mu\text{m}$  in length by 2-5  $\mu\text{m}$  in width. They also vary in shape, the oval forms with a slightly pointed end being the most common in blood smears while the curved forms with rounded ends (banana-shaped) and crescentic types are rare, more commonly seen in lung and testis smears. With Gram stain, the trophozoites give a negative reaction. They stain more clearly with Moeller's method to give a distinct blue on a reddish background. The cytoplasm appears granular and stains blue with Giemsa but is lighter at the blunt end. Darker blue granules and vacuoles may be noticed distributed throughout the cytoplasm but more towards the pointed end. The organisms stain positively on Nile blue sulphate and periodic acid Schiff (P.A.S.) reagents. A polymorphic nucleus is more commonly situated near the center and stains the usual reddish-purple with Giemsa. Oval forms may appear binucleate but great numbers of divisional forms are seen at different stages of development both intra - and extracellularly. Multiple divisions occur exemplified by cytoplasmic masses containing four to eight nuclei more or less regularly spaced. On invasion of histiocytes, a vacuole is produced where the trophozoites multiply by binary fission. As the *Besnoitia* grow the host cell nucleus divides to form a multinucleate cell and as the cyst becomes larger the cytoplasm of the host cell is flattened to form the inner (intermediate) coat of the pseudocyst with elongated strips of nuclear material along it. Depending on the number of parasites in them, the vacuoles may be quite small, measuring approximately 8  $\mu\text{m}$  in diameter during the third week of infection. Pols (1960) recorded some vacuoles of up to 50-200  $\mu\text{m}$  at nine weeks. Like *Toxoplasma*, *Besnoitia* are resistant to intracellular digestion in the host cells. A parasite induced accumulation of host cell mitochondria and endoplasmic reticulum prevents the parasitophorous vacuole containing live *Toxoplasmic* organisms from fusing with the host cell's lysosomes (Jones and Hirsch, 1972).

Outside the host cell, *Besnoitia* organisms become surrounded by a homogenous matrix around which young collagenous fibres are laid down. It is the condensation of these fibres that is thought to be responsible for the narrow rim of P.A.S and Hale positive argyrophilic material forming the boundary between the future capsule and the host cell. Fibroblasts accumulate around this collagenous mass, and the fibres eventually become hyalinized, intensely fuchsinophilic and P.A.S. positive and have interspersed mononuclear inflammatory cells. Cysts observed in cattle and goats are essentially similar to those described in the rabbits albeit much larger. The pseudocyst on average measure 100 to 600  $\mu\text{m}$  in diameter. The location of the cyst capsule outside the host cell is a characteristic feature of *Besnoitia* but the question as to the real origin of the capsule is not clarified.

## 2.2. EPIDEMIOLOGY OF BESNOITIOSIS

Species of *Besnoitia* are distributed world wide having been first reported from France in 1912 by Besnoit and Robin. In Africa, incidences have been reported from Sudan, South Africa, Angola, Rwanda, Botswana, Zambia, Tanzania, Uganda and Kenya. Pals (1960) observed cyclical variations of the disease resembling those of horse sickness, blue tongue, three days sickness and sweating sickness. *Besnoitia* species naturally affect a variety of domestic and wildlife hosts and have been experimentally transmitted to cattle, sheep, goats, rabbits, and gerbils. Morbidity in large herds is about 50 per cent. It occurs seasonally, being more common in warm wet weather. Incidences of spontaneous besnoitiosis have been noticed in Kenya (Bwangamoi, 1967; Bwangamoi, 1972; Bwangamoi *et al.*, 1989; Kaliner, 1973) suggesting that the disease may be widely distributed and insidiously spreading in the country without detection.

A case of natural besnoitiosis in a rabbit has been recorded (Mbutia *et al.*, 1993). There is no breed or age prevalence but the disease is more common in cattle over 3 years. Failure to detect infections in younger animals remains unexplained. Natural protection or passive immunity from the dam fails to account for this situation since such animals contract the disease when they grow older on enzootic farms (Bigalke, 1968). Yerusham *et al.* (1992) reported a case of clinical besnoitiosis in a 15 month old Friesian calf.

### 2.3. TRANSMISSION AND HOSTS

Infected animals remain carriers for life. Such animals develop premunity and play an important part in the epidemiology of the disease. The seasonal incidence may imply that arthropods serve as vectors. They transmit the disease mechanically from heavily parasitized cattle to susceptible ones. Several species of biting flies have been incriminated (Barrairon, 1938; Herin, 1952). Localization of many cysts in the dermis where they are easily accessible to such vectors suggests a remarkable adaptation for survival of the genus (Bigalke, 1968). Bigalke (1960) and Pols (1960) demonstrated the mechanical transmission of *Besnoitia* by *Glossina brevipalpis*, tabanid flies, *Stomoxys calcitrans*, *Culex simpsoni* and unidentified *Culex* spp. Bigalke (1960) showed that the haustellum of *Glossina brevipalpis* penetrated cyst walls without difficulty when they were allowed to feed on donor animals harbouring cysts around the tip of the tail. Other members of the sub family Stomoxyinae (*Siphona* spp. and *Haematobia* spp.) and members of the *Hippobosca* spp. may be as important, at least on account of their numbers in the range lands (Bigalke, 1968). It is, however, most unlikely that a single species of fly is responsible for the mechanical transmission of *Besnoitia besnoiti*.

Pols (1960) was unable to obtain transmission between chronic carriers and fully susceptible cattle under conditions of cohabitation and effective control measures of besnoitiosis. However, similar experiments by Bigalke (1968) showed exclusive transmission to cattle when in direct contact with carriers. Tsetse flies used in mechanical transmission experiments failed to transmit the disease 3 hours after feeding on a chronically infected bovine and there was no indication of cyclical development of parasites within them (Bigalke, 1960, 1968). He concluded that transmission operates over relatively short distances within a population of animals making biological transmission very unlikely.

Bigalke (1968) successfully infected cattle and rabbits orally with trophozoites and intranasally with cyst organisms of *Besnoitia besnoiti*. Ernst *et al.* (1968) encountered cysts of *Besnoitia jellisoni* in the vagina, on the penis and in other reproductive organs in rodents; while Nobel *et al.* (1977, 1981) encountered cysts of *B. besnoiti* in the vagina and endometrium of a cow. Whether besnoitiosis may spread naturally via the oral and nasal routes, or by coitus in intermediate hosts is yet to be clarified.

It is noteworthy that dermatotropism in besnoitiosis is expressed significantly only in the bovine. The typical chronic lesions of sclerodermatitis and alopecia are not commonly encountered in other species. Hitherto only low grade infections of cysts in the cardiovascular system, subcutis and lymphatics and some viscera have been encountered in antelopes (Basson *et al.*, 1965; McCully *et al.*, 1966). The chances of antelope cysts being ingested and transmitted mechanically are small because they are not as readily accessible as those of the bovine parasites. Visceral cysts have also been encountered in lungs of goats (Kaliner, 1973) and rabbits (Mbothia *et al.*, 1993). The mode of transmission of viscerotropic *Besnoitia* parasites is unclear,

but whatever the method, it must be quite efficient since the incidence is fairly high (Bigalke, 1968). McCully *et al.* (1966) envisaged a situation whereby periodic release of organisms occur during the chronic disease allowing blood - sucking arthropods to transmit organisms to susceptible animals either mechanically or biologically. This hypothesis has however not been proven. Frenkel (1973) reported a similar situation in toxoplasmosis where sporadic parasitaemias of low titre occur in chronic *Toxoplasma gondii* infections but its significance in natural transmission is unknown. Little or no spread of toxoplasmosis occurs from one animal to another in the acute phase, even when the animals are confined in a close place.

A coccidian life-cycle akin to that found in toxoplasmosis and other isosporoid coccidia is also considered important in the epidemiology of *Besnoitia* parasites with definitive hosts derived largely from predators of the family Felidae. Indeed, the current classification of *Besnoitia* and other cyst forming isosporoid coccidia is based on the behaviour of various stages of development in the definitive Feline hosts and the intermediate herbivorous hosts. Frenkel (1977b) groups *Toxoplasma*, *Besnoitia*, *Isospora*, *Sarcocystis* and *Hammondia* spp. together as coccidia of cats. The parasite undergoes development through the process of schizogony, gametogony and oocyst development in the intestinal epithelia of the definitive host leading to shedding of unsporulated oocysts in faeces in the case of the genera of the sub-family Toxoplasmatinae. Such oocysts sporulate outside the host and are infective to intermediate hosts. However, the specific definitive hosts are largely unknown for most species of *Besnoitia*. The domestic cat has been found instrumental to the cyclic transmission of *Besnoitia wallacei* of rodents (Frenkel, 1953, 1977b; McKenna and Charleston, 1980) through isospora oocysts. Frenkel (1977b) reported this as the only possible mode of transmission of *B. wallacei* as its

tradyzoites are not transmissible to other intermediate hosts or do so only poorly. A similar situation occurs in *Cystoisospora* and *Hammondia*.

Bwangamoi (1989a) described a new species, *Microbesnoitia leoni* isolated from the heart of a lion (*Felis leo*). This appears exceptional in that extraintestinal infection in definitive hosts has not been observed in the species of the genus *Besnoitia*. Moreover, *M. leoni* appeared to parasitize myocardial cells and were found within thin cyst walls showing a striking resemblance with *Toxoplasma*. Bwangamoi (1989a) suggested that *M. leoni* uses the lion as its intermediate host and that its final host might be found among scavenger mammals and birds.

Peteshev *et al.* (1974) demonstrated transmission of *Besnoitia besnoiti* using oocysts in pieces of two species of wild cats, *Felis libyca* and *Felis catus* but Rommel (1975) and Diesing *et al.* (1988) were unable to transmit the same species through cats, dogs and scavengers. Whether cats play any role in natural transmission of *Besnoitia besnoiti* requires further investigation. Definitive hosts for other species of the genus *Besnoitia* are unknown. Tadros and Saarman (1982) observed that carnivorism fails to account for the wide prevalence of infection with these organisms amongst strictly herbivorous animals.

The survival of cysts or oocysts of *Besnoitia* in nature should be considered in the epidemiology of the disease. Uvaliev and Baigaziev (1978) showed that cattle carcasses and skins, and a contaminated environment can be sources of *Besnoitia besnoiti* infection. Discarded skin portions harboured viable cysts for up to 30-90 days, while cysts survived for up to 100 days and 110 days in manured soils and lake water respectively. Studies by Polomoshnov *et al.* (1981) showed that unprotected cysts of *B. besnoiti* could retain their infectivity for 8 months at -20°C and 4 months at -30°C. Such cysts died in direct sunlight in 3-4 hours and in 1 minute in water



at 70°C. A variety of ordinary disinfectants killed cysts in between 2 minutes to 10 hours. Póls (1960) showed that *Besnoitia* could survive in citrated blood for 4 days and probably longer at +4°C.

### 2.3.1. Artificial transmission

Although earlier attempts to transmit *Besnoitia* using cyst suspensions by various parenteral routes and natural openings had singularly failed, Bigalke (1967), Bigalke and Naudé (1962) and Newman (1962) successfully transmitted the disease using cyst forms in rabbits and cattle. Bigalke (1967) found that rabbits developed typical besnoitiosis after having received cyst suspensions prepared from infected skin or tissues from chronically infected cattle by parenteral routes. Cattle infected in the same way developed a mild form of the disease. Earlier, sub-inoculation of blood obtained during the febrile phase of the disease showed that the disease could be transmitted from natural cases to susceptible cattle (Cuille *et al.*, 1936; Póls 1954a, 1960); proliferative forms of *Besnoitia besnoiti* in the blood were responsible for inducing the disease (Póls, 1954a). Studies by Póls (1954a, 1954b) showed that rabbits were highly susceptible to intravenous, intraperitoneal and subcutaneous inoculations of infected blood while transmission was also possible through infected subcutaneous oedematous fluid, suspensions of diseased tissues and tissue cultures.

*In vitro* studies on the parasite have been enabled by recent developments in cultivation of the parasite in cultures, embryonated eggs and storage of frozen stabilates (Bigalke, 1962; Bigalke *et al.*, 1974; Goldman and Pipano, 1983; Samish *et al.*, 1988; Shkap *et al.*, 1987; Suggs *et al.*, 1968). Samish *et al.* (1988) demonstrated the ability of *B. besnoiti* to grow in tick cells.

Work by Shkap *et al.* (1987) showed high susceptibility of vero (green monkey kidney), L<sub>929</sub> (mouse fibroblasts) and BEK (Bovine embryo kidney) cells to *Besnoitia* parasites, and they were also able to demonstrate susceptibility of laboratory animals to culture derived parasites.

### 2.3.2. Hosts.

It is not clear whether *Besnoitia* organisms found in various animal species belong to one species with different strains or are different species. The parasite has been isolated in a variety of domestic and wildlife host in many parts of the world (Soulsby, 1982). *Besnoitia besnoiti* (Marotel, 1912; Henry, 1913) causes besnoitiosis in cattle and rabbits experimentally. It was first reported in cattle in France by Besnoit and Robin (1912) but has since been reported in other parts of the world and is widespread in Africa. Strains of this species are believed to cause natural infection in antelopes and domestic goats. It has been demonstrated that infection with the bovine type confers immunity against the wildebeest type and *vice versa* (Bigalke *et al.*, 1974). However, studies by Bigalke *et al.* (1967), Basson *et al.* (1970) and Le Blancq *et al.* (1986) distinguished bovine and antelope parasites as two different strains of *B. besnoiti*. Demonstration of different banding patterns of six enzymes indirectly indicated clear genetic differences between the two strains. Studies to establish the role of the cat as a definitive hosts for *B. besnoiti* in its natural transmission (Peteshev *et al.*, 1974; Rommel, 1975) are inadequate. *Besnoitia benneti* (Babudieri, 1932) causes besnoitiosis in horse and ass. The species is less common than *B. besnoiti* of cattle. It is presumed to have a similar life cycle and distribution although no definitive host is known. Natural infection with *B. benneti* in a horse is recorded in Kenya (Bwangamoi, 1972). *Besnoitia tarandi* (Hadwen, 1922) causes reindeer and caribou

besnoitiosis in Canada and Sweden (Rehinder *et al.*, 1981). The definitive host is unknown. *Besnoitia jellisoni* (Frenkel, 1955) and *Besnoitia wallacei* (Wallace and Frenkel, 1975) were isolated in wild mice (*Peromyscus maniculatus*) in the U.S.A. *B. wallacei* presumably occurs throughout the world (Levine, 1977; Levine *et al.*, 1980). Its final host is the domestic cat which passes unsporulated oocysts in faeces. Sporulated oocysts are the only source of infection to the intermediate rodent hosts (Frenkel, 1977b). The definitive host for *B. jellisoni* is unknown. It produces an acute fatal disease in mice and rats. *Besnoitia darlingi* (Brumpt, 1913) was isolated from opossum and lizards (Smith and Frenkel, 1977). The definitive host is unknown but it has been experimentally transmitted to cats, laboratory mice and bats. Other named *Besnoitia* species include *Besnoitia panamensis* and *Besnoitia sauriana* (Garnham, 1966) isolated from two species of panamanian lizards, *Basiliscus basiliscus* and *Ameiva ameiva* (Schneider, 1965). *Besnoitia* - like parasites have also been described in avian hosts including flamingoes in Kenya (Karstad *et al.*, 1983) and a variety of other wildlife hosts. It is however noted that *Besnoitia* oocysts are not easily differentiated from other coccidian tissue cysts. The confusion that has marked the classification of isosporoid coccidia for a long time is evident in *Besnoitia* where host ranges and species specificity are yet to be clarified. Indeed, it may be that no species of *Besnoitia* parasitizes more than one genus of intermediate host, or perhaps the situation is such that only a knowledge of the intermediate hosts, vector or complete life cycle will permit identification to species as has been the case with other isosporoid coccidia.

## 2.4. PATHOGENESIS.

The pathogenesis of bovine besnoitiosis follows the general pattern of most infectious diseases producing an initial acute phase with parasitaemia, fever and general symptoms of malaise, followed by localization in particular tissues and subsequently, the chronic phase ensues. Besnoitiosis is primarily a vascular disease. It is unique in that the tissues mainly involved are the superficial connective tissue, the dermis, subcutaneous tissue, fascia, scleroconjunctiva, nasal and laryngeal mucus membranes (Bwangamoi, 1979).

Earlier studies were confined to subacute and chronic natural cases but development of methods of artificial infection (Pols, 1954a, 1954b; Bigalke, 1960, 1967; Newman, 1962) provided opportunities for closer studies. After an incubation period of 1-13 days, depending on the method of inoculation (Bigalke, 1968), a febrile reaction that persists for several days is observed. During this period, parasites appear in relatively large numbers in the blood of rabbits but only in small numbers in the blood of cattle. The parasites can also be demonstrated either extracellularly or intracellularly in the superficial lymph nodes of cattle, as well as in the subcutaneous oedematous fluid, lung, testis, liver and spleen of rabbits by microscopy or by subinoculation into susceptible animals (Basson *et al.*, 1970). It is notable that proliferative forms are not very frequent in the blood and a single parasite per microscope field may represent a very severe infection (Bigalke, 1960). Within the blood stream or lymph they invade the increased numbers of monocytes where they undergo multiplication with subsequent release into the circulation again, apparently after bursting the cell. The number of proliferative forms (Bigalke, 1968), the invasiveness and the rate of multiplication of the strain (Tadros and Saarman, 1982) are responsible for the degree of severity of the reaction. After remission of

the fever the parasites disappear from the circulation and are situated in the fibrous tissues of the skin, aponeurosis, testis, sclera and mucus membranes of the upper respiratory tract, where they actively infect histiocytic cells, and mature within about 71 days into chronic cysts (Bigalke, 1967, 1968; Basson *et al.*, 1970). What initially inhibits or initiates cyst development when proliferation has proceeded far enough is unknown but it is notable that extracystic proliferation soon ceases completely. A similar phenomenon in *T. gondii* is ascribed to the emergence of immunity (Jacobs, 1956). However, immunity is apparently not a prerequisite to the formation of cysts (Stahl *et al.*, 1966). Findings of regular parasitization mainly of small and medium sized blood vessels by proliferative organisms in the acute cases of bovine besnoitiosis (Basson *et al.*, 1970) as well as the abundance of *Besnoitia* cysts in these vessels of various antelopes and in cases of chronic bovine besnoitiosis (McCully *et al.*, 1966) point to the primary significance of vascular lesions in the pathogenesis of besnoitiosis. McCully *et al.* (1966) constantly found cysts on the heart valves and cusps of the jugular and other veins suggesting that slowing of blood flow or even stasis could predispose the tissues to infection. It is speculated that toxicity is also responsible for the formation of vascular lesions by inducing degenerative and necrotic changes that subsequently increases vascular permeability (Basson *et al.*, 1965, 1970).

The development and growth of large numbers of cysts in the skin, especially within the dermal papillae is responsible for the clinical manifestation of scleroderma in chronic bovine besnoitiosis. The granulomatous reaction and accompanying fibrosis around the cysts, hyperkeratosis and acanthosis further contribute to this manifestation. Degenerating or ruptured cysts prompt necrotizing inflammation and mild granulomatous reaction in the vicinity (Schulz,

1960). Clinically, complete recovery may occur but some degree of scleroderma and millions of viable cysts persists.

Basson *et al.* (1970) after inoculating rabbits with rabbit-passaged bovine strain reproduced a pattern of the disease different from the disease in cattle infected with recently isolated strains. In rabbits, proliferative organisms were more frequently encountered in tissues other than the endothelium. Arteries were frequently severely affected. Cysts developed rarely and active macrophages with abundant parasitic debris, eosinophils and some neutrophils were commonly present in the spleen. This indicated that the parasite lost its cyst-producing ability at high passage levels. Only a few cysts were encountered in one rabbit. A similar situation is reported in *Besnoitia jellisoni* infections in mice (Frenkel, 1965). Moreover, Pols (1960) and Bigalke (1967) found frequent uncomplicated deaths in rabbits during the acute phase of the disease.

## 2.5. CLINICAL SIGNS.

The clinical syndrome in cattle occurs generally in three stages; febrile, depilatory and seborrhoea sicca stages (Soulsby, 1982). The incubation period which may extend up to 13 days is followed by a steep rise in temperature of up to 41.6°C. The febrile period lasts for 2 - 10 days and is accompanied by photophobia, lacrimation and hyperaemia of the sclera, enlargement of superficial lymph nodes, serous rhinitis and oedematous swellings on limbs, scrotum and lower body parts. The swellings latter become tender, warm and render mobility difficult and painful. Diarrhoea may occur. Pin-point cysts appear on the sclero-conjunctiva and mucous membranes of the nasal cavity about 3 weeks after the febrile reaction. This causes acute

catarrhal or muco-catarrhal to purulent conjunctivitis and rhinitis, with the exudates sometimes being blood-tinged. The nasal mucosa becomes bright-red, swollen and crusts of the inspissated exudates form inside the nasal passages and around the nares leading to stricture. A short cough occurs following involvement of the pharynx and larynx. Encrustation also forms on the canthi of the eyes and progresses to conjunctivitis. Abortion may occur in pregnant animals at this stage. This initial stage may, however be mild with little obvious clinical alterations and such animals usually recover. The mild syndrome is more common in natural infections as reported by Pols (1960) and Bigalke and Naudé (1962).

Recovering animals proceed to the depilatory stage dominated by cutaneous lesions. The skin loses its elasticity, thickens and cracks on the flexor surfaces causing serosanguineous discharge. Depilation and wrinkling occur as a sequel to oedema, development of excessive fibrous tissue and pressure from cysts that impair nourishment to the dermis. Recumbency leads to formation of decubitus wounds and sit-fast on tension areas of the skin. Oedema subsides at this stage leaving the skin very wrinkled and the animal may resume grazing although the loss of condition is very marked and may persist for several months (Pols, 1960). Death is due to debility, exhaustion or secondary bacterial infections. This is the critical stage on which prognosis is based (Pols, 1960).

Progression into the seborrhoea sicca stage signifies chronicity. In bovine it is marked by a scleroderma where the skin is markedly thickened and rough. Most hair from the formerly oedematous areas is lost and oozing fissures strongly attract blow flies. Eventually the skin becomes very thick and folded resembling that of an elephant. Owing to secondary bacterial or fungal infections, there is a pronounced focal hyperplasia of the superficial cutaneous layers,

forming dense papilliform structures, especially in the scrotum or perineal regions of bulls. Sometimes the surviving hair forms peculiar patterns on the body resembling the markings of a giraffe, presumably associated with focal vascular disturbances (Schulz, 1960). Lymph nodes are permanently enlarged with persistence of cysts in the skin and visible mucosae. Such animals remain carriers for life (Pols, 1954a). Pols (1960) observed that bulls naturally infected with *Besnoitia besnoiti* develop a partial or complete sterility, which is often permanent.

Clinical signs observed in rabbits during the febrile stage were more prominent than those observed in either cattle, sheep or goats under experimental conditions (Pols, 1960). A well-defined thermal reaction developed and they lost weight rapidly during the febrile phase. They also developed hot, painful subcutaneous swellings, especially on the ears, head, limb, prepuce and scrotum at the peak of the reaction. Some males developed complete necrosis of the scrotum and testis. However, the skin lesions do not progress to the chronic scleroderma typical of bovine besnoitiosis.

Sheep infected artificially with the wildebeest strain (Bigalke *et al.*, 1967) developed distinct febrile reactions lasting from 6-11 days. They developed general signs of illness but all recovered. Similar signs were observed in sheep infected with the bovine strains. It is in doubt whether sheep develop the disease naturally. Infected goats (Pols, 1960) developed similar non-specific signs but one reacted more severely with oedematous swellings of the face and neck, lacrimation, mucoid nasal discharge and dyspnoea. Cutaneous lesions also developed. In a natural outbreak in goats (Bwangamoi *et al.*, 1989) infertility, abortion, neonatal deaths, alopecia and ocular cysts were observed. Cheema and Toofanian (1979) reported severe cutaneous lesion in a wild goat.



## 2.6. PATHOLOGY

The pathology in besnoitiosis has been reported by various workers both from chronic natural infections and experimental infections (Basson *et al.*, 1970; Pols, 1960; Schulz, 1960). Pols (1954a, 1954b, 1960) found no internal gross pathological lesions directly referable to besnoitiosis with the exception of cachexia. Cysts are visible as opaque whitish raised nodules (0.5 mm in diameter) in the membranes of the upper respiratory tract, the trachea up to its bifurcation into the bronchi and in the cutis, subcutis and aponeurosis of the muscles. In addition, cysts have been reported in the cardiovascular system of cattle, especially in veins of the head, neck and limbs. The intima in affected blood vessels appears granular (McCully *et al.*, 1966). Interestingly, a high concentration of *Besnoitia* cysts has been reported in the adrenals, probably due to the immunosuppressive effect of endogenous corticosteroids (Smith and Frenkel, 1977). However, pathological changes are dominated by lesions in the skin, mucous membranes, the cornea and the interstitial connective tissue of the skeletal musculature and the testis. They largely depend on the species of the parasite, the virulence of the strain, the history of its laboratory maintenance, the species of the host infected and the type of parasitized tissue (Schulz, 1960). Testes of the bull are markedly swollen and turgid in the acute stage, but in the chronic stage they are atrophied and turgid. Since acute infections are rare in nature and that reproducing typical clinical infections artificially in cattle is difficult, concise reports of pathological changes in the acute bovine disease have not been reported. Vascular invasion by the parasite is important in the pathogenesis of acute lesions in experimentally infected rabbits. The distribution of cysts in the eventual chronic state is also closely related to the cardiovascular system. The lesions are more characteristic and striking in the cutaneous locations with

formation of subcutaneous oedematous swellings, ascites, dermatitis and dermal necrosis and erosions involving the ears, nose, lips, scrotum and limbs. Sub-epicardial petechiae, necrotic orchitis and peri-orchitis has also been reported (Pols, 1960; Bigalke *et al.*, 1967). A leucocytosis accompanied by anaemia and a rise in monocytic count occurred concurrently with the rise in temperature and declined more or less with the drop in temperature (Bigalke *et al.*, 1967). Emaciation appeared in rabbits surviving for longer periods.

Pathological changes in domestic goats (Bwangamoi *et al.*, 1989; Smith and Frenkel, 1977) and wild goats (Cheema and Toofanian, 1979) are comparable to those reported for *Besnoitia besnoiti* in cattle. Masses of cysts are to be found in the skin, teats, intercostal muscles, fascia, eyelids, bulbar conjunctiva, endothelium of venules and arterioles and rarely in the lungs (Kaliner, 1973).

A less severe disease appears to develop in infections of rabbits with antelope strains of *Besnoitia*. Briefly, the most prominent lesions seen in rabbits infected with the blue wildebeest strain were peritonitis, splenomegaly, lymphoid hyperplasia, focal disseminated hepatitis, testicular atrophy, vasculitis and thrombosis (Bigalke *et al.*, 1967).

Histopathological changes also appear to be influenced by vascular lesions (Basson *et al.*, 1970). Vasculitis, fibrinoid vascular necrosis and thrombosis precede haemorrhages, oedema, degeneration, necrosis and infarcts particularly on the skin and testes. The occasional absence of parasitization in the testis of goats has led to the hypothesis that some toxin may be involved in the production of vascular lesions (Cheema and Toofanian, 1979). Vascular cysts may appear in the intima, sub-intima or adventitia eliciting little or no inflammatory reaction. Those in the intima lead to polypoid growths making the lumen narrow and irregular. Cutaneous changes

include acanthosis, hyperpigmentation, hyperkeratosis, papillary projections and focal necrosis with polymorphonuclear cell infiltration in the epidermis. The dermis and subcutis contain numerous masses of cysts, infiltration by neutrophils, plasma cells, lymphocytes and granulation tissue formation. Atrophy and necrosis of hair follicles and sebaceous glands usually accompanies cutaneous lesions. A closer look at the testicular tunics (Cheema and Toofanian, 1979) revealed many cysts, fibrosis, cellular infiltration and congestion of the tunica vaginalis and tunica albuginea of two wild goats with natural infections. Numerous cysts could be found in the epididymis, mainly within the adnexa or beneath atrophic epithelium of seminiferous tubules. No spermatogenic activity was evident in these testicles. Bwangamoi *et al.* (1989) encountered masses of cysts in the wall and lumen of veins and arteries of the pampiniform plexus obstructing blood flow and causing multiple thrombi which obliterated some vessels. Cysts were also in the interstitium and lamina propria of the epididymis and seminiferous tubules. Spermatogonia and spermatozoa were absent in almost all seminiferous tubules. Cysts have also been encountered in the alveolar walls of the lungs, lamina propria of abomasal mucosa and other organs which appear to be the preferred sites in antelope infections (McCully *et al.*, 1966; Cheema and Toofanian, 1979).

The parasitic cysts seem to have a special affinity for areolar connective tissue, preferably of the superficial layers of the skin, mucous membranes, male genital tract, skeletal musculature and the lymph nodes, lungs, vascular system, bone and perineural tissues to a lesser extent (Schulz, 1960). Bigalke *et al.* (1967) reported presence of proliferative forms in various tissues of three fatal cases of rabbits at high passage levels of wildebeest strain with a virtual absence of organisms in the skin, scrotum, testis, epididymis and pampiniform plexus, sites that abound

with organisms in bovine strains infections. Small numbers of cysts in the peripheral veins, nasal mucosa, endocardium and tendon sheaths were observed in sheep experimentally infected with the wildebeest strain.

## 2.7. IMMUNOLOGY

Although reference to immunity in besnoitiosis is scanty in the literature some observations regarding the immune response and surveillance have been made. Revelation has also been made of a remarkable similarity to the immunology of *Toxoplasma* infections. Experiments by Frenkel and Lunde (1966) provided evidence that probably a cellular immune process is dominant in hamsters infected with *Besnoitia jellisoni*. Though stable antibody titres were established during the subsequent chronic infection, they were insufficient to prevent relapse after cortisol injections. Frenkel (1967) successfully transferred specific immunity to *Besnoitia jellisoni* and *Toxoplasma gondii* in hamsters using cells of the spleen and lymph nodes. Such transfer was unsuccessful when intact lymphoid cells from irradiated immune donors were employed. Further studies by Frenkel and Wilson (1972), Hoff and Frenkel (1974) and Lindberg and Frenkel (1977) demonstrated specific cellular immunity to *Besnoitia* both *in vitro* and *in vivo*. This forms the basis of premunition in both artificial and natural chronic infections. The duration of such immunity is not known although it is rightly expected to remain as long as live cysts persist, which for all practical purposes, is life long (Pols 1960). No cross immunity or non-specific resistance to the related *Toxoplasma* is acquired. When the infection is controlled by chemotherapy in hamsters, a cell-mediated immunity is acquired in about 3 weeks (Frenkel and Wilson, 1972).

Frenkel and Lunde (1966), Kaggwa *et al.* (1979) and Wallace and Frenkel (1975) have demonstrated appreciable antibody titres in experimentally infected mice, hamsters and rabbits although they believe that such antibodies have little protective mechanisms. They were insufficient to prevent relapse after cortisol treatment during chronic infection (Frenkel and Lunde, 1966). Twelve rabbits died of acute besnoitiosis although serological tests detected high levels of humoral antibodies in these rabbits (Kaggwa *et al.*, 1979). This confirmed the findings of Frenkel and Wilson (1972) in *Besnoitia jellisoni* infections of hamsters. In their experiments, Frenkel and Wilson (1972) showed that although antibody was only slightly impaired by immunosuppressive doses of irradiation, it did not protect against besnoitiosis in irradiated hamsters. During immunosuppression, bradyzoites of *Toxoplasma* and *Besnoitia* are capable of initiating renewed tachyzoite proliferation and formation of additional cysts when immunity returns. It has been mentioned, however, that immunity is not a pre-requisite to bradyzoite formation as cysts can form in cell cultures. The limited protection conferred by humoral antibodies against besnoitiosis in hamsters is mainly due to the short periods of extracellular exposure of the *Besnoitia* organisms to the antibodies (Hoff and Frenkel, 1974). *Besnoitia* organisms multiply intracellularly and are disseminated in the bloodstream within cells, and are only briefly exposed to extracellular antibody such as when migrating from one cell to another (Frenkel, 1967). Intracellular *Besnoitia* are not affected by neutralizing antibody, as has also been shown for the related *Toxoplasma* (Sabin and Fieldman, 1948). However, in *in vitro* studies, humoral antibody enhances cyst formation in the absence of cellular mediation (Hoff *et al.*, 1977). Shkap *et al.* (1985b) detected specific precipitating IgG and IgM antibodies in sera of cattle naturally infected with *Besnoitia besnoiti* but their role in protective immunity of bovine

besnoitiosis needs further investigation.

Earlier, experimental observations of cross-protection between *Besnoitia* strains of cattle and antelopes led to the production of a partial but durable protective immunity in cattle using a live tissue culture vaccine prepared from a blue wildebeest strain of *Besnoitia besnoiti* in South Africa (Bigalke *et al.*, 1974). Cattle immunized with this wildebeest strain developed inadequate immunity that allowed subclinical infection following challenge with the bovine strain. This vaccine is yet to find widespread application outside South Africa.

## 2.8. DIAGNOSIS

Tentative diagnosis can be based on the seasonal occurrence of besnoitiosis, clinical signs and the lesions at necropsy (Pols, 1960) but examination for cysts in the sclero-conjunctiva and the anterior nares provides a fast diagnostic method in chronic infections (Bigalke and Naudé, 1962). To confirm diagnosis, skin biopsy may be taken from any lesion which show alopecia, or any other suspicious cutaneous manifestation or scleroconjunctival cysts. Histological examination readily shows the cysts in the papillary and the reticular layers of the corium and in the subcutis or the sclera. Blood and lymph node smears obtained during the early stages may show proliferative parasites (Pols, 1954b, 1960) and biological tests can be done on the highly susceptible rabbits to show the viability of the parasites. The clinical picture of cachexia and alopecia is a useful indicator of besnoitiosis (Bwangamoi, 1979).

Bigalke and Naudé (1962) and Bigalke (1968) concluded that many cases of besnoitiosis exist than could be detected by clinical and histological examination. Routine serological methods have also been used to demonstrate *Besnoitia* antibodies: Sabin-Feldman dye test (SFT) (Frenkel and Lunde, 1966; Hoff and Frenkel, 1974; Lunde and Jacobs, 1965), indirect haemagglutination test (IHA) (Krasov and Omarov, 1975; Lunde and Jacobs, 1965; Suggs *et al.*, 1968), complement fixation test (CFT) (Bigalke, 1966; Krasov and Omarov, 1975; Suggs *et al.*, 1968), indirect fluorescent antibody test (IFAT) (Frank *et al.*, 1970; Goldman and Pipano, 1983; Janitschke *et al.*, 1984; Kaggwa *et al.*, 1979; Newman, 1972; Shkap *et al.*, 1984), enzyme-linked immunosorbent assay (ELISA) (Janitschke *et al.*, 1984; Kaggwa *et al.*, 1979; Shkap *et al.*, 1984). Krasov and Omarov (1975) used *Besnoitia besnoiti* parasites as antigen for haemagglutination, immunodiffusion and complement fixation test. These authors obtained positive results but observed cross reactions with *Toxoplasma* in agreement with findings of Lunde and Jacobs (1965) of certain common antigens between *Toxoplasma gondii* and *Besnoitia jellisoni* in the indirect haemagglutination test, complement fixation test and a common precipitin line in the agar-gel diffusion test. Comparative antigenic studies suggested that an antigenically related spectrum of organisms exists with the *Besnoitia* and the *Toxoplasma gondii* RH isolate at opposite ends of the spectrum (Suggs *et al.*, 1968). The two parasites are, however immunologically different. Cross protection was unsuccessful suggesting specificity of the protective mechanism. The specificity of the IFAT was demonstrated by testing antisera obtained from animals immunized against *T. gondii*, *Besnoitia jellisoni*, *Babesia*, *Anaplasma*, *Theileria* and *Sarcocystis* using *Besnoitia besnoiti* as antigen (Frank *et al.*, 1970; Janitschke *et al.*, 1984;

Newman, 1972). No cross reactions were obtained (Newman, 1972). This contrasted sharply with the findings of Goldman and Pipano (1983) and those of Suggs *et al.* (1968) that showed cross reactions between *B. jellisoni* and *T. gondii* in IFAT although homologous titres obtained in these investigations were always higher than heterologous titres. *Frenkelia* and *Sarcocystis* have slight serological affinity to *B. besnoiti* (Tadros and Laarman, 1982). Janitschke *et al.* (1984) and Kaggwa *et al.* (1979) found IFAT superior to ELISA in diagnosis of latent infections and was species-specific with regard to *B. besnoiti* and *B. jellisoni* infections in rabbits and mice. ELISA detected antibodies to *Besnoitia besnoiti* and *Besnoitia jellisoni* (Kaggwa *et al.*, 1979) but the common antigens were not considered strong enough to make *B. jellisoni* a promising antigen in diagnosis of *B. besnoiti* infection in animals. Homologous antigen-antibody reactions always produced higher titres than heterologous reactions. Specific precipitating IgG and IgM antibodies have been detected in sera of cattle naturally infected with *B. besnoiti* but their value in diagnosis of besnoitiosis is obscure. The reliability of the various serological tests needs further evaluation in terms of their specificity and sensitivity if they are to be applied in mass surveys, although the presence of a chronic skin condition characterized by alopecia, dermatitis and reproductive problems in a farm should suggest besnoitiosis. Reliable modern prophylactic measures in besnoitiosis have not been investigated but Shkap *et al.* (1985a) have demonstrated therapeutic potential for oxytetracycline against *Besnoitia besnoiti*.



## MATERIALS AND METHODS

### 3.1. Preparation of *Besnoitia* parasites.

#### 3.1.1. Bovine *Besnoitia* parasites

The initial bovine *Besnoitia* parasites were primarily isolated from cysts of cattle with chronic besnoitiosis at the Agricultural Development Corporation (ADC) Galana ranch, Coast Province. Cattle parasite donors were identified by visual examination for the presence of cutaneous and sclero-conjunctiva cysts (Bigalke and Naudé, 1962). Locations with highest concentration of cysts were shaved, cleaned with soap and water and disinfected with 70% alcohol. Such areas were scraped using a sterile surgical blade (Swann Morton Ltd, Sheffield, England) until bleeding occurred and the scrapings collected in sterile bottles containing phosphate buffered saline (PBS, pH 7.2) to which 200 units/ml of benzylpenicillin sodium (DAWA Pharmaceuticals Ltd, Nairobi), 200  $\mu\text{g/ml}$  streptomycin sulphate (Mac's Pharmaceuticals Ltd, Nairobi) and 50  $\mu\text{g/ml}$  Nystatin (Mycostatin sterile powder, Squibb<sub>TM</sub>) (Bigalke, 1962) had been added. The scrapings were crushed in a mortar and pestle (Bigalke, 1967) in the field laboratory to free parasites from cysts, suspended in 20 ml PBS and passed through Whatman filter paper No. 91 (Whatman Ltd, Maidstone, England) in a funnel to separate parasites from the larger cell debris. The resulting filtrate was dispensed into sterile centrifuge bottles. Portions of the filtrate were centrifuged at 2,000 rpm for 15 minutes. The supernatant was discarded and the resulting pellets pooled and finally re-suspended in PBS to form the inoculum. Motility was used as an indicator of viable parasites. An estimate of live parasite concentration in the inocula was determined by the use of an improved Neubauer

haemocytometer (Assitent, W. Germany). In all cases smears made from the inoculum were air dried, fixed in absolute methanol (Narcolabs Enterprises, Rotterdam, Holland) for 5 minutes, stained with a 10% Giemsa solution for 45 minutes and then examined for *Besnoitia* parasites (X400 magnification). Other portions of infected skin scrapings were transported in PBS containing antibiotics under chilled conditions in a flask and processed in a similar manner at the Veterinary Laboratories, Kabete. The material was preserved in 1% dimethylsulfoxide (DMSO) as stock inoculum.

### 3.1.2. Caprine *Besnoitia* parasites

*Besnoitia* parasites were isolated from cysts of a goat suffering from chronic besnoitiosis at the Bachuma sheep and goats farm, Coast Province. The goat was euthanized with an intravenous injection of 10 ml Euthatol (RMB Animal Health Ltd, Dagenham, England) and subsequently skinned aseptically to expose the subcutaneous fascia. The mid-line of the abdomen was shaved, washed and disinfected with 70% alcohol before making the incision. Shiny white sand-like cysts could be seen embedded in the fascia over the abdomen, especially over the limbs and back. The cysts were harvested by cutting off sections of the fascia aseptically using a pair of thumb forceps and a surgical blade. The cysts were then suspended in PBS. The material was preserved overnight at 4°C and processed as described above.

### 3.2. **Experimental Animals.**

The rabbits used in these experiments were the New Zealand White breed aged 5 months and weighing 2kg. They were obtained from coccidia-free stock bred at Kabete. Both sexes were

used. They were housed singly in wire cages and provided with clean water, rabbit pellets (Rabbit pellets, Belfast Miller Ltd, Nairobi) and vegetable supplement *ad libitum*.

Local goats, between 6 and 8 months old were obtained from besnoitiosis-free area and therefore assumed not to be infected with *Besnoitia*. Physical examination was done to determine absence of the obvious cysts in the sclera-conjunctiva. They were housed in a concrete floor stockade under tick and fly-free conditions at the Veterinary Laboratories, Kabete. They were provided with clean water *ad libitum*, red oat hay, commercial feed (Unga cubes, Unga group (K) Ltd, Nairobi) and Maclick mineral supplement (Cooper (K) Ltd, Nairobi) and dewormed with Nilzan plus (Cooper (K) Ltd) on introduction into the stockade. Three weeks before infections, faecal samples were collected *per rectum* for worm egg counts and Giemsa stained blood smears prepared for haemoparasite screening. All the goats were carefully washed with steladone acaricide (Kenya Swiss Chemicals, Nairobi) two weeks before infection. One week prior to infection, a meticulous visual examination was carried out for any obvious cutaneous lesions or other skin condition. Blood samples containing the anticoagulant ethylenediaminetetraacetate (EDTA) were collected at the same time to provide the baseline haematological data. Experimental infections were carried out at exactly five weeks after the goats were introduced into the stockade.

### **3.3. Infection of Experimental Animals.**

In all twenty-seven animals were involved in this experiment. These included 11 goats and 16 rabbits. All animals were labelled and placed in two groups each comprising both rabbits and goats. A third group consisting of two rabbits (R126 and R128) and two goats (Gc1 and

Gc2) were kept as controls. All experimental animals were infected with *Besnoitia* parasites suspended in PBS using different infection routes.

Control rabbit R126 and goat Gc1 were injected with 20 ml of sterile PBS subcutaneously while rabbit R128 and goat Gc2 were left uninfected.

The rabbits were infected with *Besnoitia* parasites as follows;

Cyst organisms of bovine *Besnoitia besnoiti* were used to infect eight rabbits, R101, R103, R104, R105, R111, R123, R148, and R150 using  $0.1 - 0.25 \times 10^6$  parasites. Rabbits R101 and R103 were infected intravenously and intraperitoneally respectively while the rest were infected subcutaneously.

Proliferative organisms of bovine *Besnoitia* goat-passaged parasites were used to infect rabbits, R154, R156 and R161. Ten milliliters of heparinized blood drawn from infected goats GHe3, GHe4 and GHe5 at the peak of the thermal reaction was injected intraperitoneally into rabbits R156, R161 and R154 respectively.

Cysts organisms of caprine *Besnoitia* parasites were used in the infection of three rabbits, R106, R108, and R109 using  $0.25 \times 10^6$  parasites. All were infected subcutaneously.

Goats were infected with *Besnoitia* parasites as follows:

Cyst organisms of bovine *Besnoitia* parasites were used to infect four goats GHe1, GHe2, GHe3, and GHe4 using  $0.6 \times 10^6$  parasites. All were infected subcutaneously. Goats GHe3 and GHe4 were treated with Furaxol in drinking water (50 gms /liter) for two weeks pre-infection to try and delay the infection (Pols, 1954, 1960).

Two goats, GHe5 and GHe6, were transfused intravenously with 100 ml heparinized blood collected during the acute phase from goats GHe3 and GHe4 respectively.

Cyst organisms of caprine *Besnoitia* ( $0.6 \times 10^6$  parasites) were used in subcutaneous infection of 3 goats, GHo1, GHo2, and GHo3.

#### 4. Animal observations.

##### 4.1. Clinical Examination

Infected animals were regularly examined for signs of besnoitiosis. Rectal temperatures were taken every morning during the period immediately after infection and throughout the acute phase of the disease. In addition blood and lymph node smears were taken. The smears were air dried, fixed in absolute methanol for 5 minutes and stained with 10% Giemsa stain for 30 minutes. The smears were then examined under oil emersion (X1000) for *Besnoitia* parasites, and differential white cell counts undertaken on blood smears. Temperature was recorded between 5 days pre-infection and the last day of febrile reaction.

##### 4.2. Haematology

Haematological analysis were carried out on EDTA blood samples taken from all the experimental animals at weekly intervals throughout the study period. Rabbits were bled from marginal ear vein and the goats from the jugular veins using 18G x 1½" disposable needles (Terijekt disposable needles, TSK) and allowing blood to flow freely into sterile 5 ml bijou bottles containing EDTA. The EDTA blood was drawn into microhaematocrit tubes (length 75 mm, inner diameter 1.1-1.2 mm, outer 1.5-1.6 mm), spun in a microhaematocrit centrifuge (hemofuge, Haraeus, Christ) and packed cell volume (PCV) values read on haematocrit tube reader (Hawsley, England). Blood smears prepared weekly as described above were used to

study differential white cell counts in peripheral blood using a manual laboratory cell counter. A total of 200 white cells were counted on every smear and the percentage calculated for each cell type represented (Schalm *et al.*, 1975). Blood collected into 5 ml bijou bottles containing EDTA at the same intervals were used to establish total white cell counts in a Coulter counter (Model ZM) after lysing erythrocytes using zap-o-globin (Coulter Electronics (K) Ltd.). The total number of cells for each cell type was obtained by multiplying the total white cell count by the percentage differential count for the particular cell type (Schalm *et al.*, 1975).

### 3.4.3. Gross Pathology and Histopathology

Comparative necropsy and histopathological studies were carried out on the animals that died at various stages of the experiment and those that were sacrificed at the end of the study period. After a thorough gross examination of all the systems, specimens from grossly infected areas, various organs and tissues, including skin from the several sites and preference areas like the pampiniform plexus, epididymis and the testis in males and the adrenal glands were collected in 10% buffered formalin. These specimens were embedded in paraffin wax, cut at 5-7 $\mu$ m thickness with a rotating microtome (LEITZ 1512), stained with Haematoxylin and Eosin (H/E), Periodic Acid Schiff (P.A.S) and Giemsa stains (Basson *et al.*, 1970) for cysts and other pathological lesions, fungal lesions and for extracellular and intracellular trophozoites.

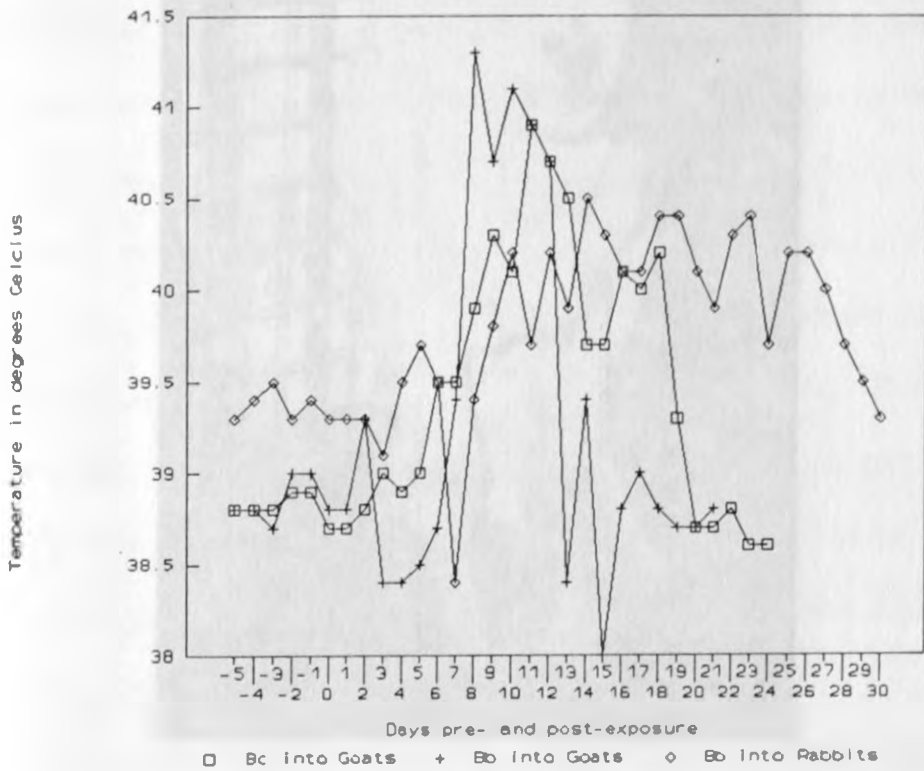
## RESULTS

### 1. Clinical and Haematological findings

#### 1.1. Infection of goats with bovine cyst organisms

Observations were made on four goats infected with bradyzoite forms from crushed bovine cysts of *Besnoitia besnoiti*.

Acute reactions were obtained in the goats after an incubation period that varied from 8 to 13 days when temperature rise was detected. Because of the fluctuating body temperatures and the influence of ambient temperature, only responses exceeding 39.5°C in goats were regarded as significant in the study. Pyrexia persisted for 5 to 11 days and fluctuated between 39.8°C and 41.1°C (Figure 1, Appendix 1). In 2 to 3 days before the temperature rise, the superficial lymph nodes nearest to infection sites become enlarged. Later other lymph nodes had a variable degree of swelling and some became very oedematous as the disease progressed. Dejection, trembling and anorexia were the early ill appearance. This was accompanied by lacrimation, rhinitis, swelling of the face, oedema of the cornea, eyelids, ears, lips, palate, the tail and the vulva at, or shortly after the peak of the febrile reaction which occurred on the 10<sup>th</sup> day of infection. Thereafter there was an extension of these signs especially oedema which extended to the neck, brisket and the legs and became very pronounced leading to a general state of anasarca. In two cases the heads became very enlarged and heavy causing a lot of distress and recumbency (Figure 2). The ocular and nasal discharges changed from serous to mucopurulent due to secondary bacterial infection causing conjunctivitis, rhinitis and pneumonia. The latter precipitated a situation of respiratory distress, sometimes severe with occasional moist coughs.



**FIGURE 1:** Mean body temperature (°C) in goats and rabbits infected with bovine *Besnoitia* strain by days pre-infection (-) and post-infection; and type of inoculum: Bovine *Besnoitia* cysts (Bc) and bovine *Besnoitia* proliferative organisms (Bb).





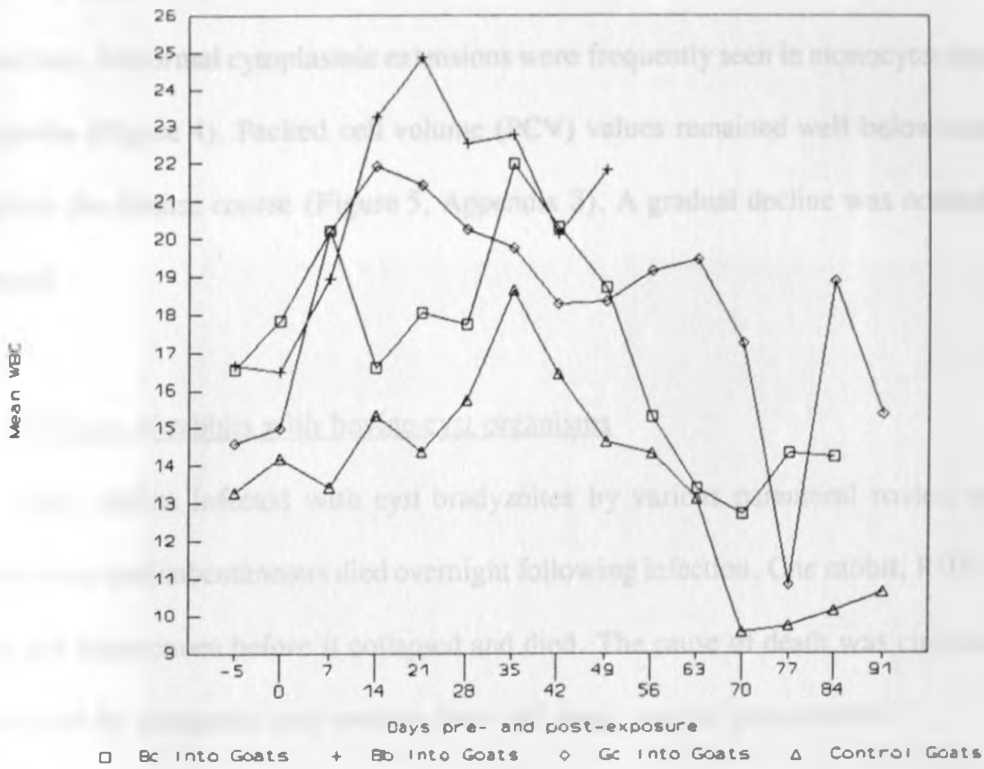
**Figure 2:** Goat infected with *B. besnoiti* cyst showing oedema of the head, eyes, ears, lips, legs and brisket.

Hyperaemia of the scleral blood vessels was a constant clinical sign. Occasional petechial and ecchymotic haemorrhages appeared on the palate and the vulva.

Two goats, GHe1 and GHe2 were recumbent, anorexic and developed sub-normal temperature before they died on day 21 and 20 post- infection, respectively. Two other goats, GHe3 and GHe4 had the primary acute reactions subsiding 4 and 5 days after temperature fell back to normal, but GHe4 had been treated with sulphadimidine to prevent imminent death at this stage. These four goats were generally weak, emaciated and had rough hair coats as oedema subsided. The hyperaemia on the sclera also waned but the conjunctiva developed rough appearance, a sign of early cyst development. In GHe4, alopecia developed over the neck, flanks, the medial aspects of the limbs and the posterior parts of the body. Thirty days after pyrexia, goat GHe3 had visible *Besnoitia* cysts on the conjunctiva, but this goat died 50 days post-infection due to extreme emaciation. GHe4 was sacrificed at the end of the study period.

During the pyrexia phase, extracellular parasites were occasionally demonstrated on smears of oedema fluid, blood and lymph aspirates.

The rise in the leucocytic counts revealed two peaks. An initial peak occurred concurrently with the rise in temperature while a second much higher peak occurred after the febrile reaction coinciding with the period of severe clinical disease (Figure 3, Appendix 2).

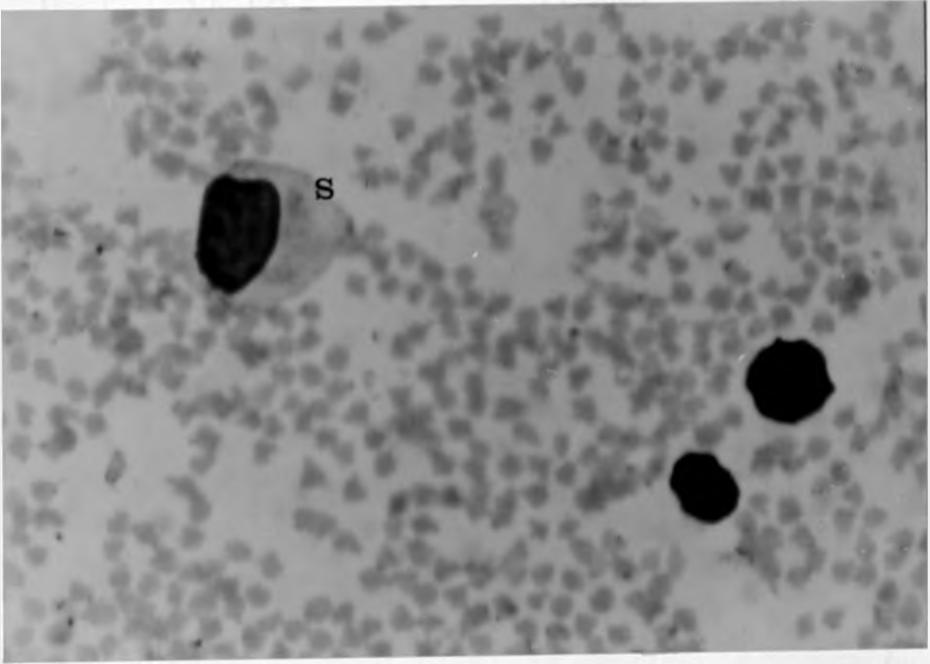


**FIGURE 3:** Leucocyte cell counts in goats infected with bovine *Besnoitia* cyst (Bc), bovine *Besnoitia* proliferative (Bb), caprine *Besnoitia* cyst (Gc) organisms and controls.

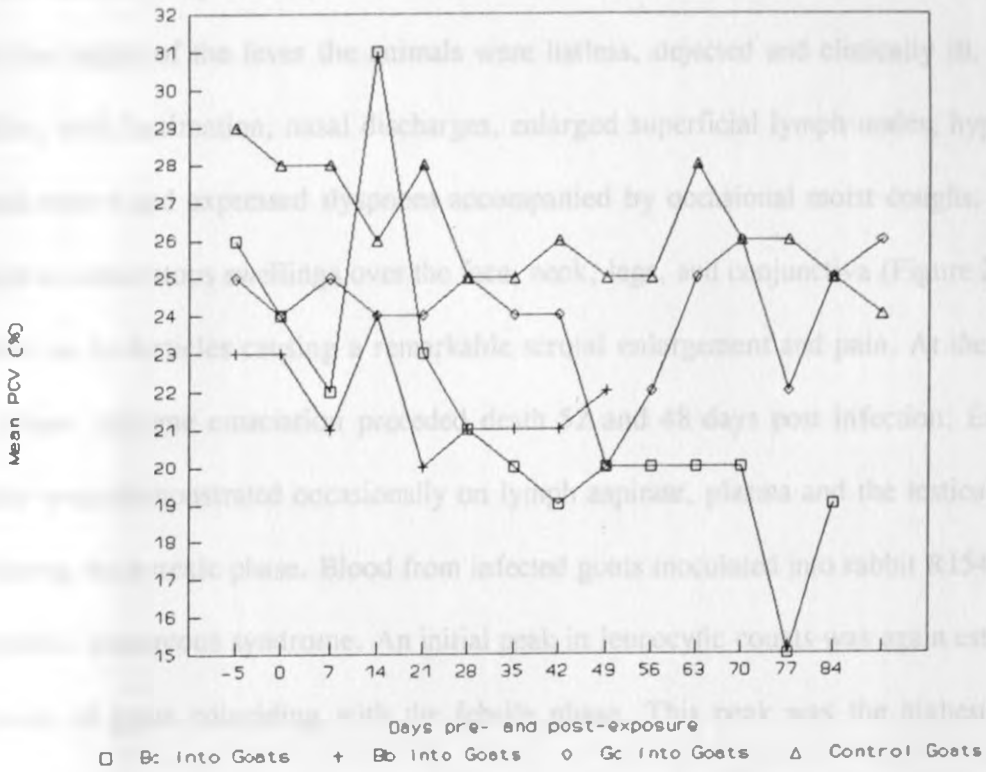
Leucocyte counts fell as the acute clinical manifestations subsided but remained considerably higher than counts from control animals. Basophils and eosinophils were least represented across the leucocytic spectrum but there was an abundance of monocytes and lymphocytes. Abnormal cytoplasmic extensions were frequently seen in monocytes appearing like pseudopodia (Figure 4). Packed cell volume (PCV) values remained well below control values throughout the disease course (Figure 5, Appendix 3). A gradual decline was noticed as disease progressed.

#### 4.1.2. Infection of rabbits with bovine cyst organisms

Eight rabbits infected with cyst bradyzoites by various parenteral routes, intravenous, intraperitoneal and subcutaneous died overnight following infection. One rabbit, R105, developed pyrexia and hyperpnoea before it collapsed and died. The cause of death was circulatory failure characterized by congested and swollen liver and lungs seen at post-mortem.



**Figure 4:** A Monocyte with pseudopodia-like cytoplasmic extension (S).



**FIGURE 5:** Packed cell volume (PCV) values in goats infected with bovine *Besnoitia* cyst (Bc), bovine *Besnoitia* proliferative (Bb), caprine *Besnoitia* cyst (Gc) organisms and controls.

#### 4.1.3. Infection of goats with bovine proliferative forms (trophozoites)

An acute syndrome, similar to that described in goats infected with cyst organisms was expressed by two goats infected with proliferative organisms. The incubation period was reduced from 11 days to 7 days and pyrexia lasted for 6 to 8 days reaching a peak of 41.4°C (Figure 1). At the height of the fever the animals were listless, dejected and clinically ill. They were trembling with lacrimation, nasal discharges, enlarged superficial lymph nodes, hyperaemia of the conjunctiva and expressed dyspnoea accompanied by occasional moist coughs. Both goats had severe oedematous swellings over the face, neck, legs, and conjunctiva (Figure 2). Oedema extended to the testicles causing a remarkable scrotal enlargement and pain. At the end of the acute phase, extreme emaciation preceded death 52 and 48 days post infection. Extracellular parasites were demonstrated occasionally on lymph aspirate, plasma and the testicular oedema fluid during the pyrexial phase. Blood from infected goats inoculated into rabbit R154 developed the classical anasarca syndrome. An initial peak in leucocytic counts was again established in this group of goats coinciding with the febrile phase. This peak was the highest and more prolonged in duration than that obtained following infection with bovine cyst organisms (Figure 3). A second peak was barely noticed before the two goats died in extreme emaciation and debility at the acute clinical phase 48 and 52 days post-infection. Similarly, an abundance of monocytes and lymphocytes was apparent. Packed cell volume (PCV) values obtained in this group were the lowest amongst the three study groups of goats (Figure 5). A gradual fall in PCV was noticed again and continued until the animals died.

#### 1.1.4. Infection of rabbits with bovine proliferative forms (trophozoites)

Three rabbits were infected with proliferative forms of bovine *Besnoitia* organisms. All developed similar clinical signs during the febrile stage. A well defined temperature reaction was noticed in all the three rabbits after an incubation period of 7 days (Figure 1). Because of the fluctuating body temperatures and the influence of ambient temperature, only responses exceeding 39.7°C in rabbits were regarded as significant in the study. Fever (39.6°C-41.2°C) persisted for 15 days and longer in one rabbit. During the fever, the animals were listless, anorexic, and lost weight rapidly. This was followed by development of hot painful subcutaneous oedematous swellings on the ears, eyes, vulva, head and limbs in both males and females. In males there was prepuccial, testicular and scrotal oedematous swellings. In addition, there was testicular reddening with progression to complete necrosis of the scrotum and testis. In one case, the scrotum opened into an ugly wound causing a lot of distress. Rabbit, R154, developed congestion, oedema, skin necrosis and gangrene on the ears which latter sloughed off to stumps (Figure 6). Serosanguineous exudate from the wounds formed into crusts and sitfasts that bled easily on the ears. Congestion and gangrene also occurred on the lower parts of the limbs, the malanges, and over the nose. Hyperaemia of the sclera and lacrimation developed in all the animals within this period. Serous to mucoid nasal discharges and marked respiratory distress were also noted.

A severe skin reaction in two cases (R154 and R156) developed into scleroderma (often seen in cattle when depilation occurs) over areas that were previously oedematous. One rabbit (R156) was extremely emaciated and died on the 42nd day post-infection. Another (R154) was sacrificed on the 73rd day to alleviate further suffering while the third (R161) survived until the

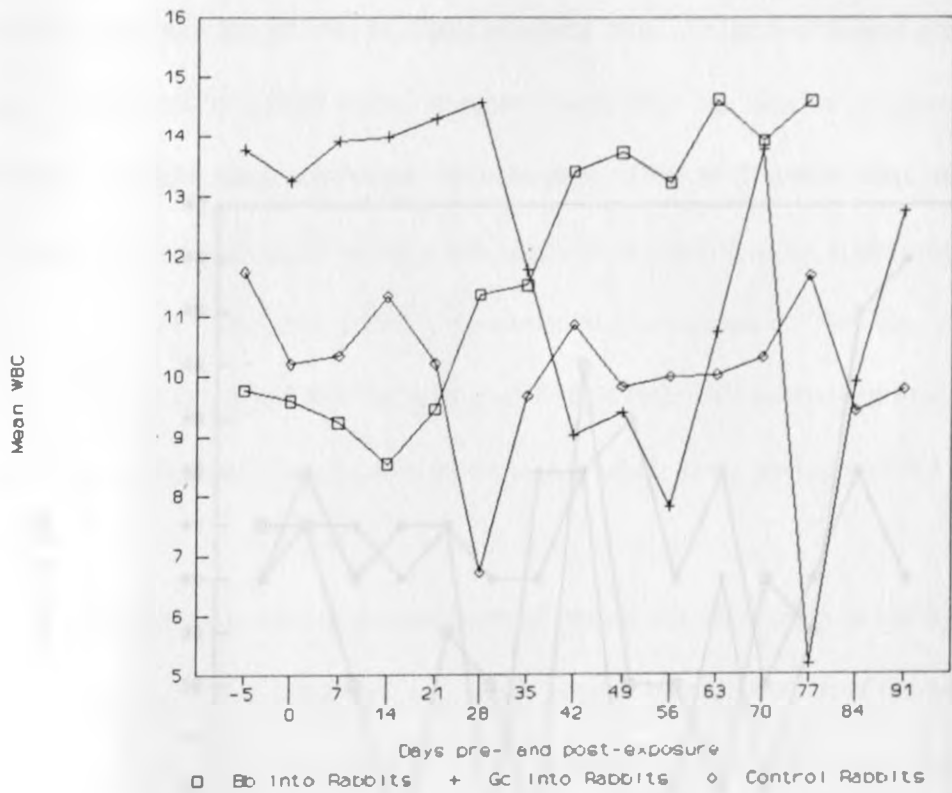




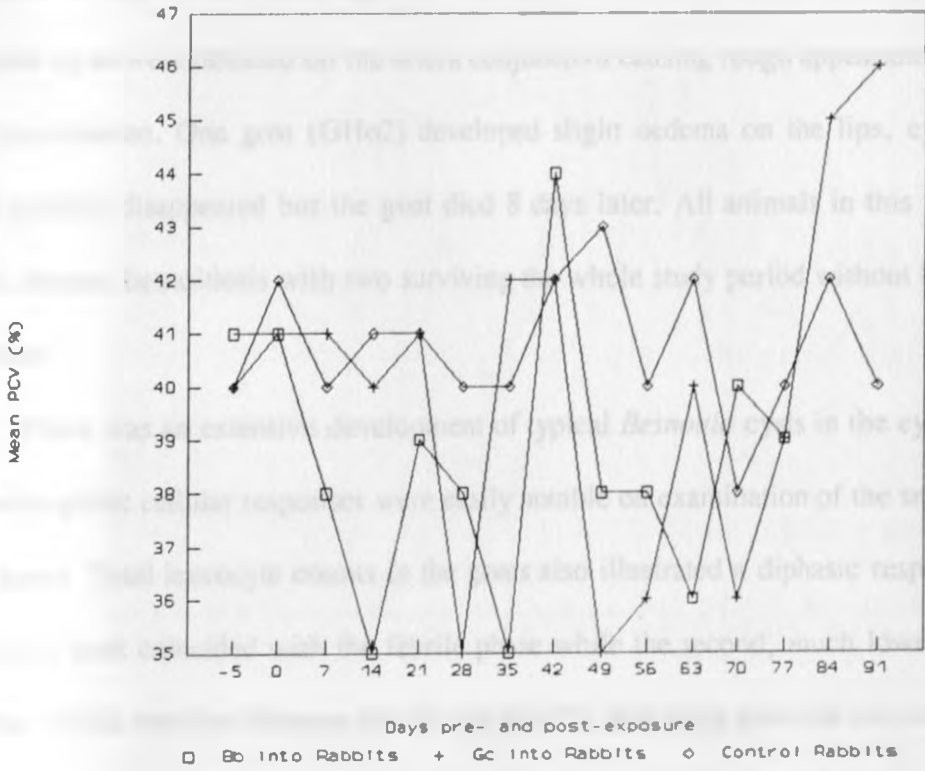
**Figure 6:** Rabbit, R154 infected with bovine *Besnoitia* proliferative forms showing congestion and necrosis (→) of the ears.

nd of the study period.

Anaemia became apparent in blood smears at about the peak of the clinical signs. The anaemia was characterized by hypochromatic macroerythrocytes. Extracellular parasites were present on stained blood smears and wet mounts of oedema fluid and the serosanguineous skin exudates from the ears at the same time. Leucocytosis was a characteristic blood picture in all blood smears taken during the inflammatory reaction. This was also confirmed by total leucocyte counts (Figure 7, Appendix 2). The initial rise in leucocyte counts was delayed upto around the middle of the febrile reaction (day 21) and maintained a steady rise far above control counts throughout the course of the study. Only one peak was noticed. Packed cell volume (PCV) dropped on day 7, two days before temperature rise and remained generally below control values throughout the disease course (Figure 8, Appendix 3).



**FIGURE 7:** Leucocyte cell counts in rabbits infected with bovine *Besnoitia* proliferative (Bb), caprine *Besnoitia* cyst (Gc) organisms and controls.

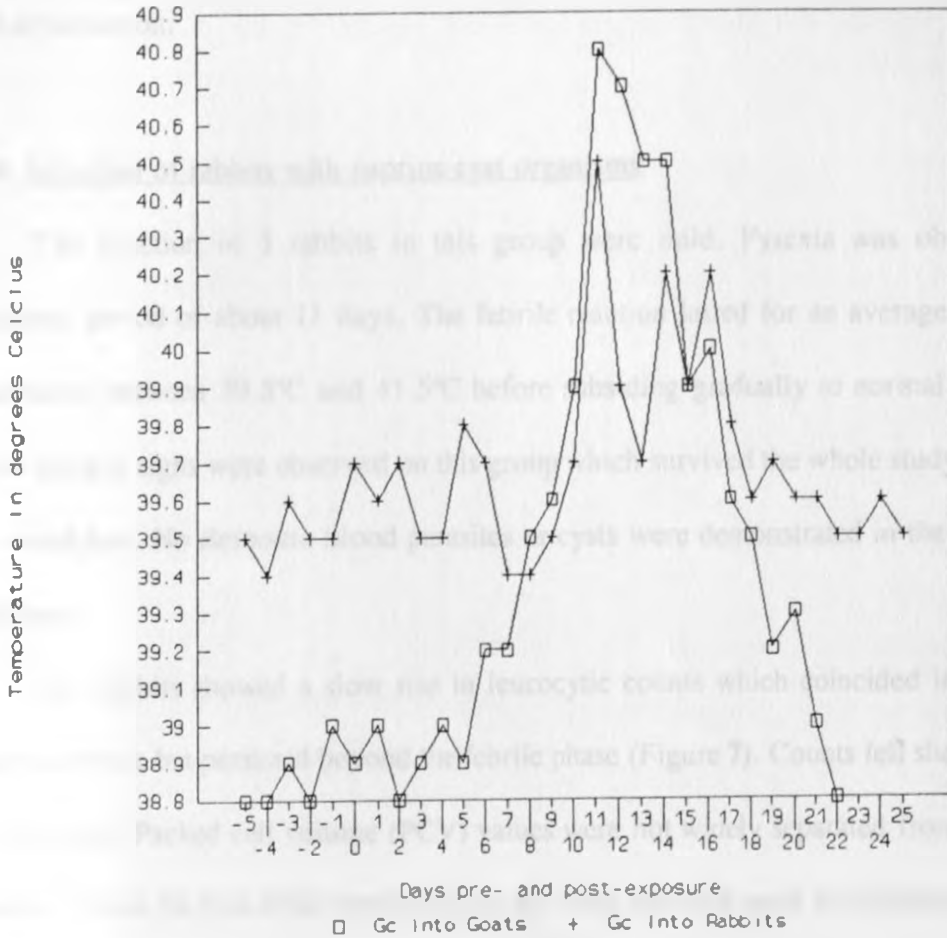


**FIGURE 8:** Packed cell volume (PCV) values in rabbits infected with bovine *Besnoitia* proliferative (Bb), caprine *Besnoitia* cyst (Gc) organisms and controls.

#### 4.1.5. Infection of goats with caprine cyst organisms

Three goats infected with caprine cyst bradyzoites expressed pyrexia, anorexia and superficial lymphadenopathy. A thermal reaction was noted 11-12 days after infection. Fever persisted for an average of 8 days, rising to a peak of 41.2°C and subsiding gradually to normal (Figure 9). Enlarged lymph nodes regressed soon after the decline of pyrexia. One animal developed a slight nasal discharge for two days. Two to 3 weeks after the febrile phase, *Besnoitia* cysts were detected on the sclera conjunctiva causing rough appearance in the eyes and slight lacrimation. One goat (GHo2) developed slight oedema on the lips, eyelids and vulva which quickly disappeared but the goat died 8 days later. All animals in this group developed typical chronic besnoitiosis with two surviving the whole study period without losing their body condition.

There was an extensive development of typical *Besnoitia* cysts in the eyes. Monocytosis and neutrophilic cellular responses were easily notable on examination of the smears made from these goats. Total leucocyte counts in the goats also illustrated a diphasic response (Figure 3). The initial peak coincided with the febrile phase while the second, much lower peak appeared after the febrile reaction between day 49 and day 77. The latter peak did not coincide with any clinical manifestations in this study group of goats. Leucocyte counts beyond day 77 remained above control values but without a regular pattern.



**FIGURE 9:** Temperature response (°C) in goats and rabbits infected with caprine *Besnoitia* cysts (Gc) before (-) and after infection. Day 0 is the day of infection.

Packed cell volume values in the goats remained below control values but higher than those in other test groups of goats (Figure 5). This was the situation throughout the experimental period in the goats. The differences between these and control values was reduced after the 11th week of infection.

#### 1.6. Infection of rabbits with caprine cyst organisms

The reaction in 3 rabbits in this group were mild. Pyrexia was observed after an incubation period of about 11 days. The febrile reaction lasted for an average of 5 days with temperature between 39.8°C and 41.5°C before subsiding gradually to normal (Figure 9). No further clinical signs were observed on this group which survived the whole study period in good body condition. No *Besnoitia* blood parasites or cysts were demonstrated in the three rabbits in this group.

The rabbits showed a slow rise in leucocytic counts which coincided initially with the temperature rise but persisted beyond the febrile phase (Figure 7). Counts fell sharply after week of infection. Packed cell volume (PCV) values were not widely separated from control values in rabbits within the first three weeks (Figure 8). After the third week the response was irregular but generally below control averages. This was interrupted by a sharp and transient peak between weeks 4 and 7, and a steady rise after week 10.

## 4.2. Gross Pathological lesions

### 4.2.1. Infection with bovine cyst organisms

Two goats (GHe1 and GHe2) died during the acute phase on day 21 and day 20 respectively. Their carcasses were extremely emaciated as shown by gelatinous body fat degeneration and had rough body hair coat. A blood-tinged mucopurulent discharge from the nose was noted in one case.

On opening the skin, there was congestion of subcutaneous blood vessels. Anasarca of the head, neck and limbs was a major lesion. Hydrothorax, ascites, and hydropericardium were noticed. The lymph nodes, liver, lungs and spleen were enlarged and swollen due to oedema. There was lymphadenopathy and hypertrophy of the heart muscles involving the ventricles. Haemorrhages were seen in the superficial lymph nodes, lungs, spleen, the sub-epicardium, the mucous membranes of the palate and the vulva, and brain meninges. There was pneumonia involving the apical lobes of the lungs with red and gray hepatization in one case. Carcasses of goats GHe3 and GHe4 that died or were sacrificed in the subacute or chronic phase were only grossly emaciated. *Besnoitia* cysts were grossly noticeable only on the conjunctiva of one goat, GHe3.

One rabbit, R105 had congested and swollen liver and lungs. No significant gross lesions were detected at postmortem in the other rabbits except hyperaemia at the site of injection. This included one rabbit given 3 days pre-infection treatment with 2% sulphadimidine in drinking water.



#### 4.2.2. Infection with bovine proliferative organisms

Two goats (GHe5 and GHe6) and one rabbit (R156) in this group died during the acute phase reactions between day 42 and day 52. Rabbits R154 and R161 were sacrificed in the chronic phase. Anasarca and cachexia were the major gross pathological lesions in both goats and rabbits. One goat, GHe5, showed petechiae haemorrhages and notable grayish thrombi under the skin of the limbs. Haemorrhages were encountered in the lungs in one rabbit. No gross signs of *Besnoitia* cysts were seen in any of the animals.

#### 4.2.3. Infection with caprine cyst organisms

One goat, GHo2, died in the course of the study while the other animals in this group, goats GHo1 and GHo3, and rabbits R106, R108 and R109 were sacrificed at the end of the study period, day 80. Post mortem examination was performed on all the animals. All carcasses were in good body condition.

Typical *Besnoitia* cysts were seen in all the goats. The cysts appeared as white sand-like particles embedded in the subcutaneous and intermuscular fascia around the neck, limbs above the carpal joints and stifle joints, the gluteal muscles, the back along the spine, over the visceral aspect of the thoracic wall and rarely over the abdominal surface. In the same areas white streaks were observed in the muscles which were assumed to be cysts. Mild oedema of superficial lymph nodes was seen in goat GHo2. A large perforating ulcer of the rumen was also seen in GHo2. This ulcer was unrelated to besnoitiosis and was the cause of death.

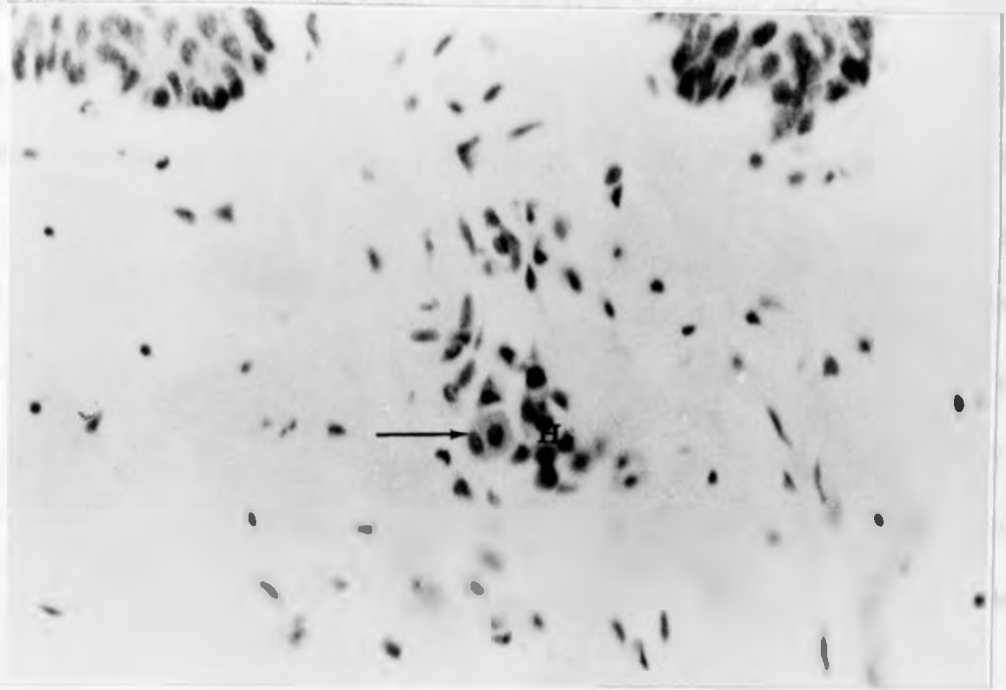
Tiny petechiations were observed on the superficial muscles of the shoulder and back in rabbit R108. No significant gross lesions were seen in rabbits R106 and R109. Samples were collected from grossly infected areas and other organs and tissues of the body for histopathological examination from all the animals during the post-mortem examination.

### **4.3. Microscopic lesions**

#### **4.3.1. Infection of goats with bovine cyst organisms**

##### Acute stage lesions

The primary lesion in goats GHe1 and GHe2 that died during the clinical acute phase was evidently confined to the vascular system. A state of pronounced hyperaemia and oedema was observed. Many of the blood vessels, from the smallest capillaries, arterioles and venules contained hypertrophied and dislodged endothelial cells. This was most prominent in vessels within the loose connective tissue of the skin, subcutis and the superficial skeletal muscle tissues. Similar observations were made in blood vessels within various other organs such as the brain, pancreas, lungs, trachea and the liver. Vasculitis was frequently encountered with arterioles showing various degrees of fibrinoid necrosis and perivascular oedema. Haemorrhages within the vessel walls were also frequently seen. Arterioles in some cases contained microthrombi and capillaries were encountered clogged with large multinucleated (2-3 nuclei) cells. Swollen histiocytic cells, often in clusters of 4-6 and containing parasitic vacuoles, were frequent in the loose connective tissue resembling epithelial glandular cells (Figure 10). These were often closely associated with the arterioles and capillaries manifesting a perivascular cellular reaction in diffuse or linear arrangements. Lymphocytes, plasma cells, fibroblasts and few



**Figure 10:** H/E, x400: A section of the skin showing a binucleated swollen histiocyte (→) in the midst of other histiocytic cells and lymphocytes (H).

polymorphonuclear cells were also encountered in this characteristic perivascular cell infiltration. Perivascular cell infiltration was most striking in blood vessels within the cutaneous and the superficial skeletal muscular tissue. Free proliferative organisms in moderate to fairly large numbers were seen in the capillaries, arterioles and lymph spaces between loose connective tissue fibres especially in the immediate perivascular space in the dermis and subcutis.

Hyperplasia and parakeratosis of the epidermis were noticed in a few areas of the skin, notably around the ears, face and tail. These were areas previously swollen with oedema. The affected skin manifested an increase in the number of cells of the stratum spinosum and thickness of the stratum corneum with or without nuclear material remnants. Subcutaneous muscle fibres had undergone hyaline degeneration characterized by muscle fibre fragmentation and infiltration with macrophage phagocytic cells and large histiocytes with *Besnoitia* parasites. Affected muscle fibres stained deeply eosinophilic with H/E stain. Focal disseminated pyo-granulomatous epidermal and superficial dermal lesions and sub-corneal microabscesses were often encountered following secondary bacterial and fungal infections.

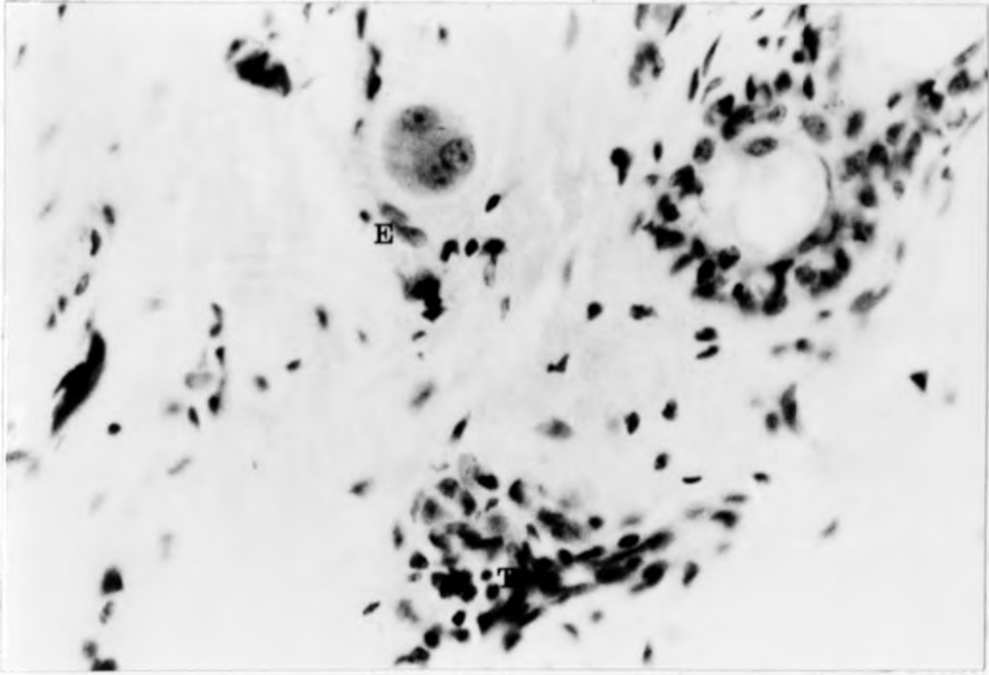
Acute reaction was seen in the lungs causing interstitial pneumonia characterized by hyperaemia and oedema, inter-alveolar epithelialization and infiltration by large mononuclear cells in the alveolar septa. Submucosal lymphocytic infiltration and mucosal erosions in the trachea were noticed in one case.

Kidneys were generally degenerated having tubular epithelium staining intensely eosinophilic. Casts of proteinaceous excretory material were lodged in the tubules. Perineuritis was noticed in one case affecting nerve fibres within the adventitia of the urinary bladder. Perineural inflammatory cells were seen in the perineurium.

### Subacute stage lesions

Two goats in this group, GHe3 and GHe4, died in the clinical subacute phase. The histopathological changes were marked by mild vascular hyperaemia and perivascular oedema. The perivascular inflammatory cell reactions persisted in the cutaneous loose connective tissues, superficial muscles and a few other areas in the viscera. Most characteristic was the presence of developing *Besnoitia* cysts at various maturation stages (Figure 11). Hypertrophied endothelial cells and clusters of hypertrophied histiocytic cells were frequently encountered in the loose connective tissue, especially in the dermis and subcutis part of the skin. The tongue, submucosa of the nose and the upper part of the trachea, the skin of the scrotum, the testicular tunics and trabeculae, the superficial skeletal muscles, the lungs and the vessel walls including the capillary vessels in the cerebral cortex of the brain had degenerative vascular changes. Microthrombi were present in the small capillaries, arterioles and venules. The walls of some of these vessels revealed mild angiopathy and partial asymmetric fibrinoid necrosis. This was mainly seen in vessels of the skin and testis. No perivascular cellular reaction was seen in the brain and the meninges were not inflamed.

Some intravascular and extravascular free organisms were noticeable and distributed in various tissues. Occasionally some histiocytic cells contained *Besnoitia* parasitic vacuoles (with 4 to 8 or more *Besnoitia* organisms) and were multinucleated, representing very young developing cysts (Figure 12). One cell had at least 5 nuclei. Accurate counts were difficult where parasites or nuclei were lying on top of each other. Many young differentiated cysts were present (size 72 $\mu$ m to 97 $\mu$ m) with an inner eosinophilic cytoplasm containing increased numbers of nuclei (3 to 7) and *Besnoitia* organisms. The nuclei were often arranged in arch forms around



**Figure 11:** H/E, x400: A section of the skin showing a developing multinucleated *Besnoitia* cyst (more advanced than figure 10) within the loose connective tissue. A cluster of swollen histiocytic cells and other inflammatory cells forming a perivascular cuff around a blood capillary (T) and extravascular *Besnoitia* parasites (E) can be seen.

**Figure 12:** H/E, x100: A young cyst in the subcutis showing two large nuclei, a parasitic vacuole (A) and a fibrous capsule around it. No noticeable cellular host reaction.



the periphery of the cell resembling Langerhan's giant cells although other random arrangements were also encountered. The largest developing cyst had 2 to 13 or more nuclei and measured 154 $\mu$ m to 283 $\mu$ m across the widest axis. The pseudocysts were surrounded by a thick connective tissue capsule that varied in diameter depending on the maturation stage of the cyst. The thinnest capsule measured was 15 $\mu$ m and the thickest was 42 $\mu$ m wide. Although the perivascular mononuclear cell reaction was still conspicuous especially in the skin, ear, testis and in other connective tissue, there was little or no inflammatory reaction around cysts that were strictly in the connective tissue. Some, however, looked degenerated and contained pyknotic nuclei with or without an inflammatory cell reaction. *Besnoitia* cysts appeared to prefer the loose connective tissue around the tail, nose, lips, muzzle, tongue, teats, testicles and scrotum. Cysts were also encountered in the dense connective tissue of a carpal joint capsule in one case. In this case, the serous spaces in the joint capsule were lined by hypertrophied cells and a cyst was encountered within the lumen while another was loosely attached to the endothelium of a muscular artery. In goat, GHe4, *Besnoitia* cysts and several hypertrophied histiocytic cells were found in the sclera of the eye.

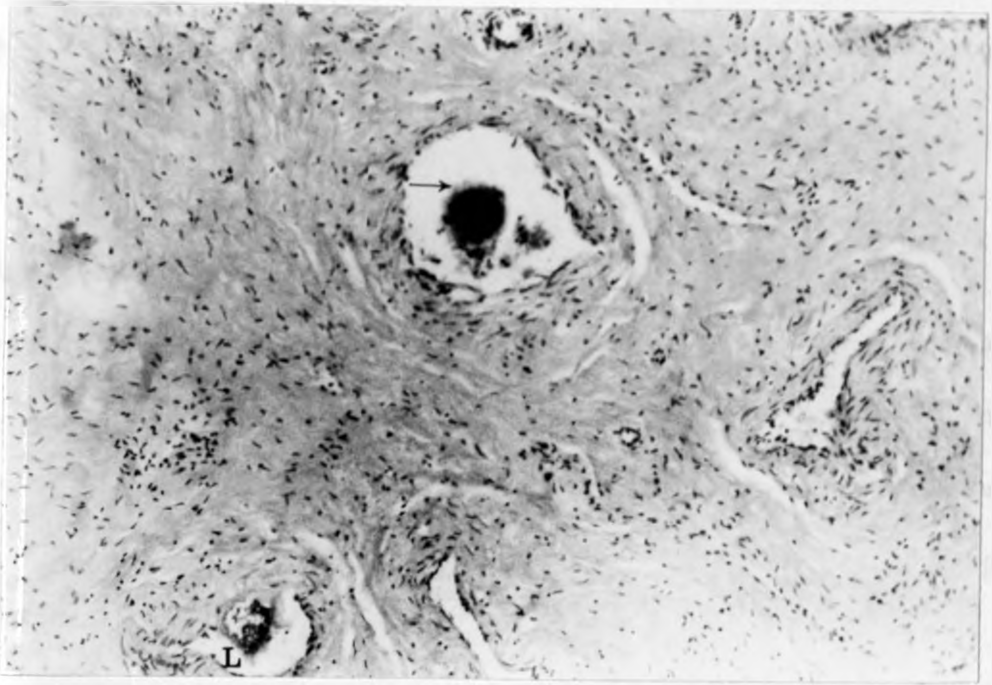
Acanthosis, parakeratosis, ballooning degeneration of epidermal and mucosal cells, necrocytosis with polymorphonuclear cells, cellulitis and scleroderma were encountered in skin and mucus membranes in the affected areas. Folliculitis and degeneration of the sebaceous and sweat glands were frequently seen. In one case, sweat gland ducts were lined by hypertrophied cells and some were evidently parasitized with *Besnoitia* organisms. Subcutaneous skeletal muscles and adipose tissue occasionally showed extensive degeneration or had undergone myositis and panniculitis with large macrophages containing eosinophilic phagocytic vacuoles.

edema was prominent in some cases. Such reactions were notably absent in the deeper laying muscles and the myocardium. Localized pyo-granulomatous inflammation and coagulative necrosis of the epidermis as a result of bacterial or fungal infections and an increase in mononuclear cells were apparent in sections of the skin from various parts of the body.

Testicular lesions were very remarkable in the male. Coagulative necrotic orchitis and epididymitis were accompanied by infiltration with large mononuclear cells and occasional polymorphs in the tunica albuginea and the connective tissue trabeculae. Hypertrophied endothelial cells were encountered in the capillaries, arterioles and venules and several differentiated *Besnoitia* cysts were found firmly attached to the endothelium of the vessels. One *Besnoitia* cyst was encountered free in the lumen of an artery apparently after breaking off from the endothelial attachment (Figure 13). Infestation with cysts in the tunica albuginea was marked and sometimes large colonies of cysts were encountered. One such colony had at least 30 cysts of different sizes (Figure 14). Some enlarging adjoining cysts had fused walls producing conglomerates. Spermatic cells were degenerated producing amorphous eosinophilic bodies within the seminiferous tubules. Sometimes sections of seminiferous tubules showed complete coagulative necrosis. However, sections of the epididymis showed clumps of degenerated spermatozoa within the lumen.

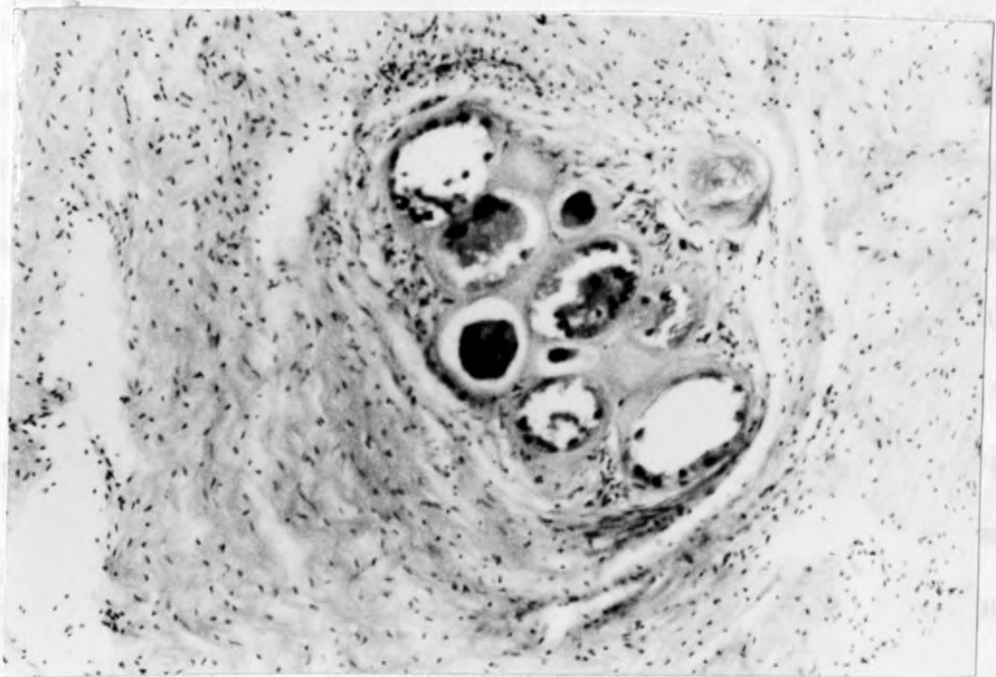
There was a notable absence of *Besnoitia* organisms and cysts from brain and other visceral organs in all animals in this group. General cloudy swelling in the renal tubules and intra-lobular cloudy swelling and vacuolar degeneration of hepatocytes encountered in these animals were not accompanied by any indication of parasitization in the kidney and liver. In one case suspect infected histiocytic cells were seen within the connective tissue in the muscular





**Figure 13:** H/E, x400: A *Besnoitia* cyst embolus (→) in a muscular artery within the testicular tunics of a buck. Another *Besnoitia* cyst on the intima of a blood vessel protruding into the lumen (L).

**Figure 14:** H/E, x100: Testicular besnoitiosis. A colony of cysts within the tunica vaginalis.



layer of the reticulum part of the forestomachs. A few of these cells appeared degenerated and homogeneously basophilic.

#### 4.3.2. Infection of rabbits with bovine cyst organisms

Various tissues were taken from the eight rabbits infected with bovine *Besnoitia* cyst organisms. Sections of the liver, lung and spleen showed hyperaemia on histopathology in four cases, R105, R111, R123 and R150. One rabbit, R123, showed mild oedema around the infection site. No changes were seen on sections of the heart, brain, lymph nodes, nasal septum, female reproductive organs, testicular and cutaneous tissue.

#### 4.3.3. Infection of goats with bovine proliferative organisms

##### Subacute stage lesions

Goats GHe5 and GHe6, infected with bovine proliferative trophozoites died at the clinical subacute phase of besnoitiosis. Lesions in the two goats were generally similar to those of the previous group infected with *Besnoitia* cyst organisms, albeit lower in disease severity. Vascular changes of hyperaemia and oedema were conspicuous in the skin, superficial skeletal muscles, and to a lesser extent in the deeper tissues and visceral organs. Angiopathy and partial fibrinoid necrosis of vessel walls were occasionally seen. Hypertrophied and parasitized endothelial cells of the small capillaries were frequently encountered resulting in complete obliteration of the lumen. Perivascular mononuclear cell cuffing was again characteristic mainly comprising of histiocytic cells and numerous lymphocytes and plasma cells. Foci of similar cellular reactions were also encountered in the lymph spaces within the loose connective tissue and away from any

blood vessel particularly in the dermis, subcutis and superficial skeletal muscle. These foci mingled with single parasites or congregations of upto 5 or more suspect extravascular piriform *Besnoitia* organisms (Figure 11). Piriform organisms also occurred intravascularly often attached to the endothelium. Isolated histiocytic and endothelial cells were evidently parasitized and had parasitic vacuoles and were multinucleated, representing the very early stages of young *Besnoitia* cysts (Figure 15).

*Besnoitia* cysts at different maturation stages occurred in the mucosal and cutaneous connective tissue of the dermis and subcutis, although their presence was moderate compared to the recipients of cyst organisms. Cysts varied from 77 $\mu$ m to 249 $\mu$ m in diameter. Inflammatory cell reactions around differentiated cysts were absent. Parasitization in this group of animals also showed an apparent preference to the loose connective tissues around the face, ears, tongue, tail, testicular tunics and interstitium and the scrotum. Various skin sections showed sclerodermitis with increased eosinophilia and oedema in the dermal and subcutaneous connective tissue fibres. Sections of subcutaneous adipose tissue were also frequently seen at various degrees of necrosis. Superficial dermatitis with sub-corneal microabscesses were constantly seen in the skin of the scrotum. Various other secondary skin reactions observed were folliculitis, focal dermal and epidermal purulent dermatitis, acanthosis, hyperkeratosis, degenerative or caseous necrosis of glandular adnexa, fungal dermatitis and subacute purulent conjunctivitis.

Many *Besnoitia* cysts were found in the testicular tunics and in the interstitium of the epididymis; others occurred in the arteries, arterioles and veins anchored in the endothelium and protruding into the vascular lumen. Occasional vasculitis characterized by cellular infiltration in the walls by mononuclear cells was seen. Diffuse mononuclear cell infiltration, marked by



**Figure 15:** H/E, x400: An early stage of cyst development showing a parasitic vacuole (→) without cellular host reaction.

large macrophages, was notable in the scrotum producing extensive perivascular cuffing and connective tissue atrophy. Atrophy of testicular parenchyma and aspermatogenesis accompanied focal pyo-granulomatous orchitis characterized by giant cells, large mononuclear cells, few polymorphs and bacterial colonies or necrotic areas.

Cellular inflammatory reaction was found involving the subcutaneous muscle and adipose tissue layers, but differentiated *Besnoitia* cysts were only seen in the interstitium of the subcutaneous skeletal muscles in one case. These *Besnoitia* cysts attracted inflammatory cells mainly lymphocytes and macrophages. One *Besnoitia* cyst in goat GHe5 was completely necrotized and ruptured forming an eosinophilic granulomatous inflammatory reaction. In goat GHe6, a severe *Besnoitia* cyst infestation was observed affecting a muscle tendon and the tendon sheath in an area below the carpal joint of the fore-limb (Figure 16). Conglomeration of cysts with fused walls were observed while other developing cysts were lined up along the associated arterioles of these tendons. This was accompanied by a marked inflammatory cell response. Superficial muscles were often degenerated and some had undergone lysis. Numerous macrophages were encountered in these areas. In some cases, only ballooned macrophages and oedema were evident where muscles had been necrotized. Such lesions were not observed in the deeper muscles, except one case with general myocardial degeneration without any inflammatory cellular response. No *Besnoitia* cysts or free parasites occurred in the heart.

One large and differentiated cyst was observed in the tunica media of a hepatic vein. This was surrounded by lymphocytes and macrophages within the wall of the hepatic vein (Figure 17a and 17b). It had numerous bradyzoites that had pushed the cytoplasm of the cell into an eosinophilic ring around the periphery of the cell. This cyst measured 289 x 308 $\mu$ m and had a

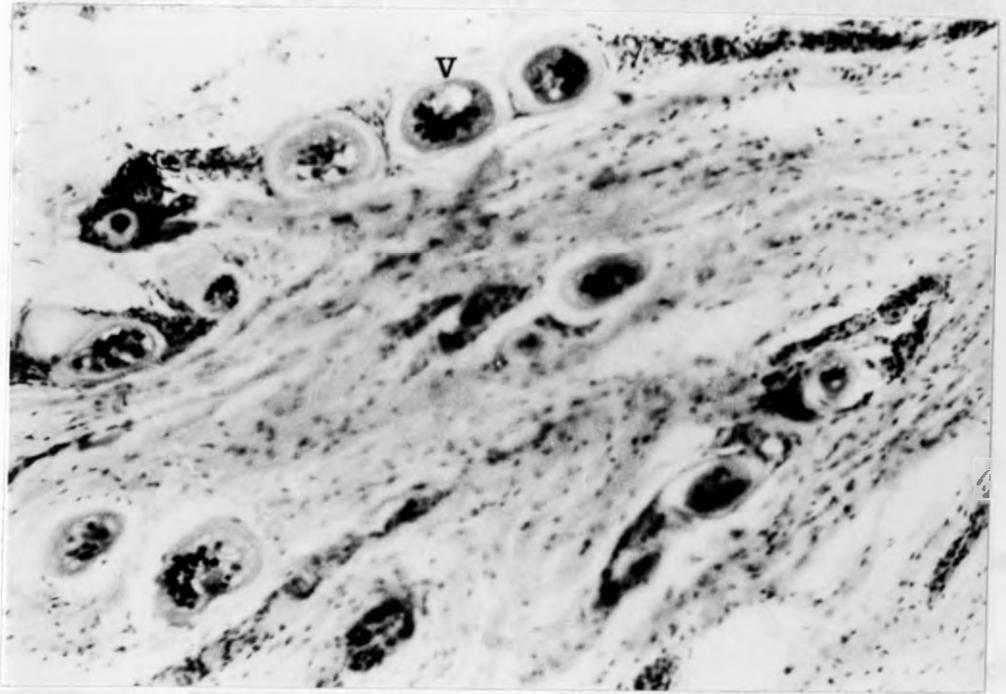
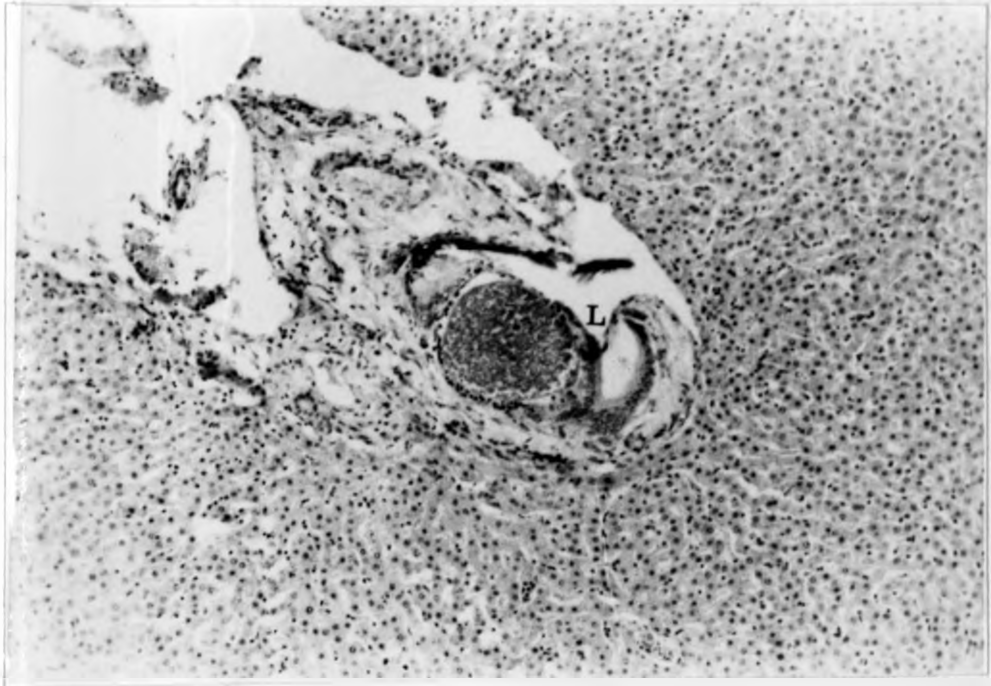


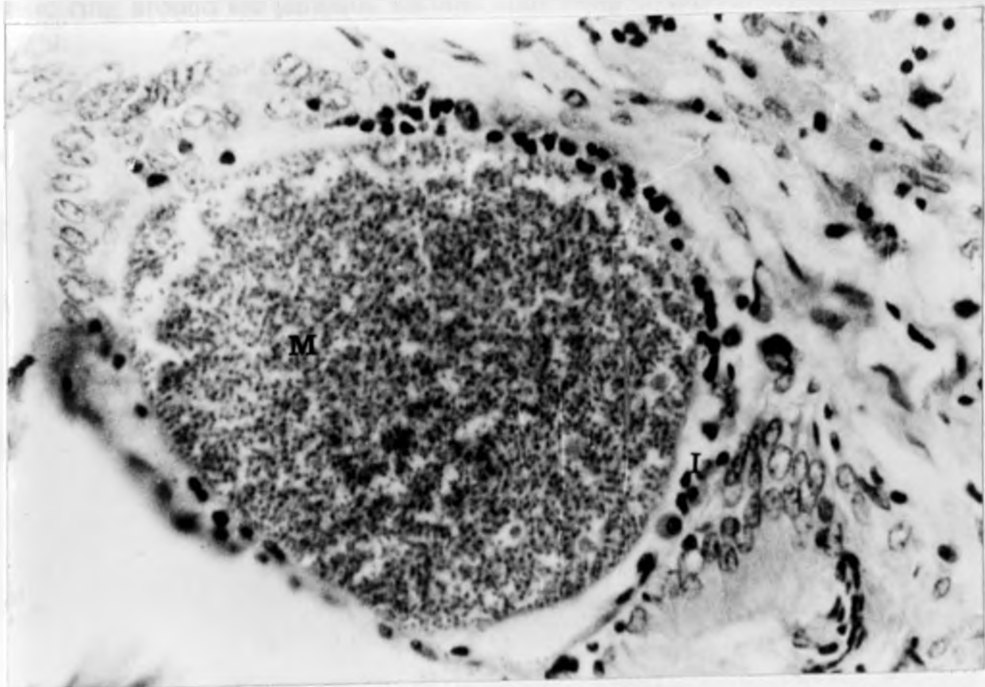
Figure 17a  
and the left  
Figure 17b

**Figure 16:** H/E, x100: Muscular tendon besnoitiosis. Severe parasitization of the leg muscle, tendon and tendon sheath. Some cysts are lined along a blood vessel (V) and have provoked infiltration by mononuclear cells.



**Figure 17a:** H/E, x100: A large mature *Besnoitia* cyst in the liver, occluding the hepatic vein and the bile duct (L).

**Figure 17b:** H/E x 400: A higher magnification of cyst in figure 17a showing masses of bradyzoites (M) and a ring of lymphocytes and plasma cells around the cyst (I).





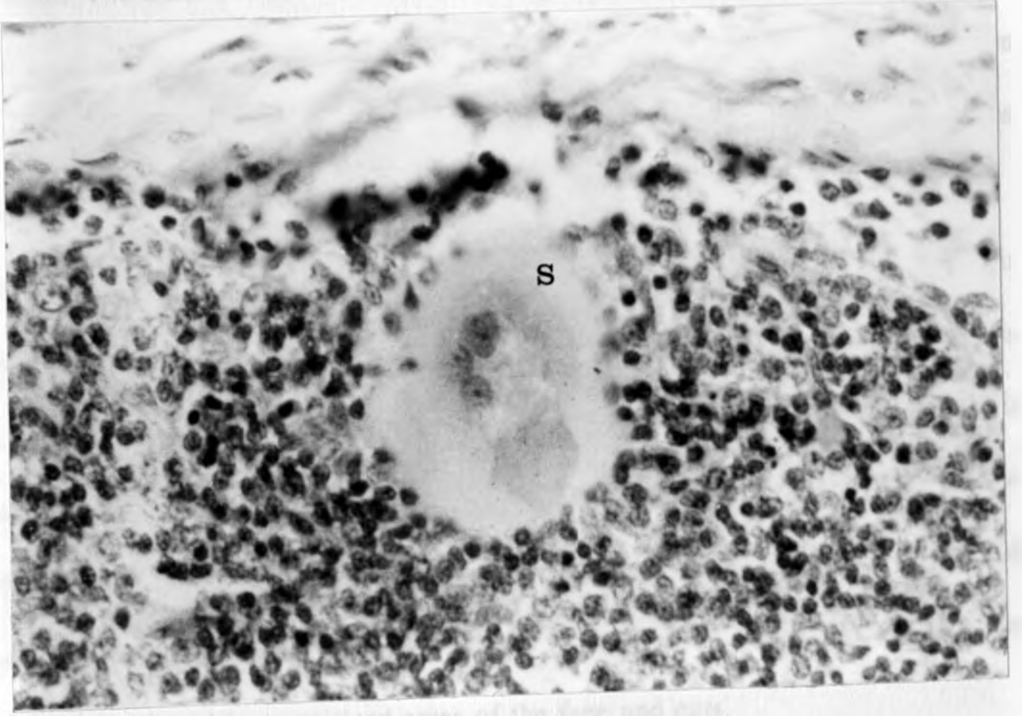
in capsule of  $20.73\mu\text{m}$  in thickness. A submandibular lymph node in the same animal had a large *Besnoitia* cyst within the lymphoid cortex and numerous clusters of hypertrophied histiocytic cells with large nuclei deep in the lymphoid tissue (Figure 18). It was not clear whether these cells were actually parasitized or not. General vacuolar degeneration and hyperaemia of the kidney and the liver cells, and hyperaemia and oedema of the lungs were encountered. Other internal organs and the gastrointestinal tract were apparently free of *Besnoitia* cysts or cellular reactions.

### 3.4. Infection of rabbits with bovine proliferative organisms

#### Acute and chronic stage lesions.

Lesions in the rabbits which had received the proliferative organisms of bovine *B. besnoiti* were very similar to those found in the goats in that they were marked by development of numerous chronic *Besnoitia* cysts. Much of the cytoplasm in the parasitized cells was reduced to an eosinophilic ring around the parasitic vacuole containing numerous bradyzoites. Varying numbers of hypertrophied basophilic nuclei were usually encountered in an arch arrangement at the pole of the cell. Thirteen nuclei were counted in one cell. The pseudocysts were surrounded on the outside by eosinophilic fibrous capsules of varying thickness depending on their maturation levels. Cysts were generally spherical in shape although incomplete shapes were encountered where cysts were inflamed, necrotized or calcified. Cysts varied in diameter from  $4\mu\text{m}$  in small cysts to  $310\mu\text{m}$  in the large cysts. The most parasitized tissues were the nose, nasal septum, lips, muzzle, ears, eye sclera, tail, testicular connective tissue, scrotum, and the ampiniform plexus. These cysts in the rabbits elicited pronounced inflammatory cell reaction around them consisting mainly of lymphocytes, large macrophages and giant cells. The



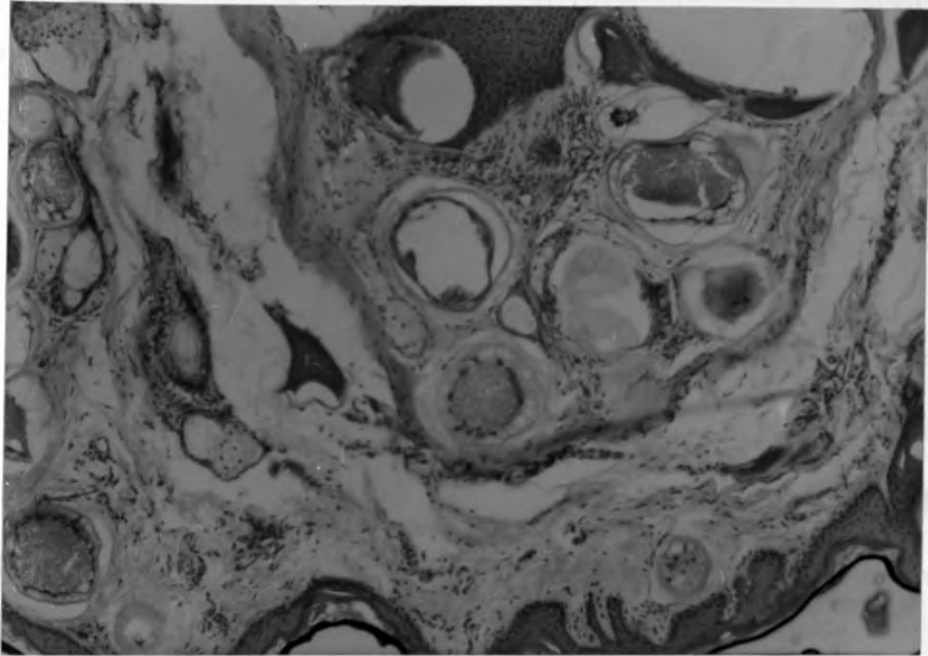


**Figure 18:** H/E, x400: A *Besnoitia* cyst (S) within the lymphoid cortex of a sub-mandibular lymph node.

mononuclear cell reaction also involved the arteries and, to a lesser extent the veins indicating generalized arteritis and phlebitis. A few arterioles and capillaries had thrombi. In one rabbit, R154, a generalized arteriosclerosis was encountered in peripheral medium and large arteries, the aorta and the heart endocardium. Generalized myocardial degeneration was noticed in the heart of this rabbit. The other two rabbits had localized myocardial degeneration. Eosinophils were rarely seen in the localized inflammatory cell reactions but they were a prominent feature in myositis, diffuse interstitial pneumonia and in the hyperaemic medium and smaller arteries of the scrotum and testicular tunics of these rabbits.

*Besnoitia* cysts were found in the sweat glands, the large arteries of the tactile hair follicles (Figure 19), the perichondrium of the ear cartilage and nasal septa, subcutaneous adipose tissue and in one instance the base of the iris at its angle with the cornea in the eye. This was very close to the retina. Large colonies and septate *Besnoitia* cysts encountered in these areas attracted extensive inflammatory mononuclear cell infiltrations. Necrotized and calcified cysts were also constantly observed with intense cellular reactions. Hyperaemia and oedema was notable in internal organs and in parasitized areas of the face and ears.

Marked congestion, haemorrhages and oedema apparently preceded pronounced localized necrosis of the skin of the tail, ears, muzzle, nose, lips and scrotum where open wounds and necrotic casts were encountered. Necrotic, calcified and ruptured cysts were noted amongst the necrotic mass in the scrotum of R156. Sections of sloughed-off epidermal layers were also encountered on the lips, muzzle and ears. These lesions were invariably associated with underlying numerous colonies of *Besnoitia* cysts and extensive tissue damage. Septate cysts, conglomerations, necrotized and calcified *Besnoitia* cysts were frequently seen within the large



**Figure 19:** H/E, x100: Intense parasitization of a tactile hair follicle in the skin of the lips showing severe destruction of the artery.

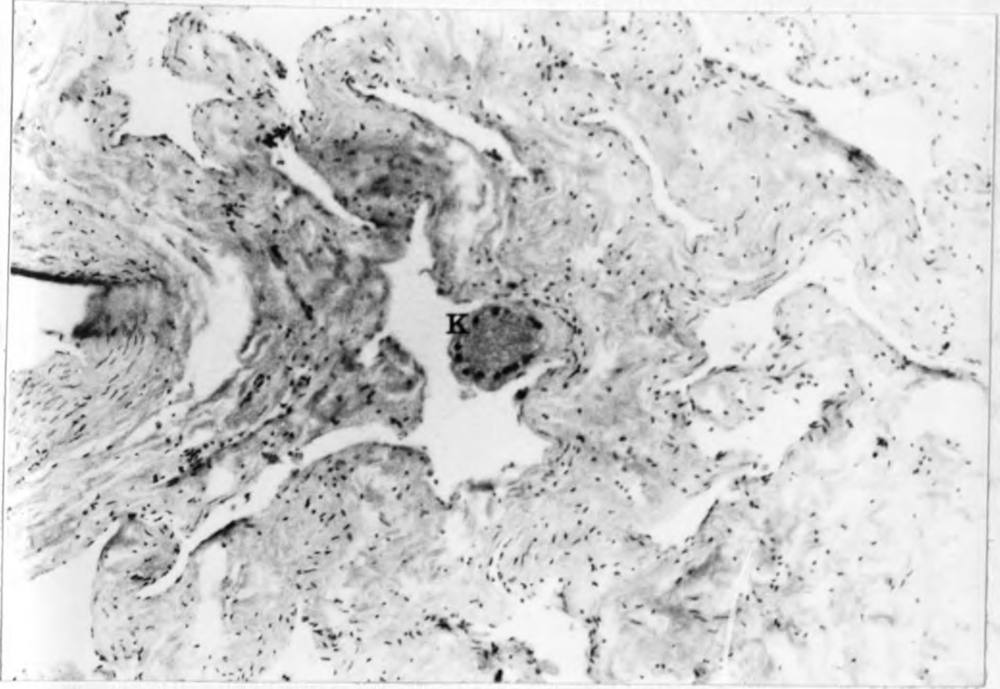
cyst colonies in the muzzle, external nares, nasal septum, the lips and the ears. The periosteum of the facial bones and the perichondrium of the ear and nasal cartilage contained cysts often causing pressure atrophy on these tissues. Pressure atrophy of the mucosal epithelia, sweat glands, sebaceous glands and the epidermis in some areas was also notable. Hyperplasia and vacuolar degeneration were marked in the glandular adnexa. Parasitized hair follicles were atrophic or necrotized altogether. Occasionally cysts were seen loosely anchored to the arterial endothelium or even ruptured into the lumen of cutaneous arteries. Cysts were also located in the media or the adventitia or combinations of these layers of many cutaneous arteries causing bulging of the endothelium or extending away from the endothelium instead. Nuclei in the myofibrils of the arterial wall were often swollen apparently in the process of degeneration. Cysts in the glandular skin adnexa of the scrotum attracted a predominantly eosinophilic cellular response. Skin from the neck, the back, flanks, the abdomen and the gluteals was only very mildly affected. Most sections from these areas contained neither the cysts nor the characteristic cellular reactions. There were no *Besnoitia* cysts seen in the epidermis.

Acanthosis and parakeratosis of the skin were frequent findings, especially in areas that were heavily parasitized. Sebaceous glands revealed lesions of hyaline degeneration and sometimes necrosis. Possible parasitization of glandular cells or duct epithelial cells could not be verified. Foci of secondary fungal dermatitis, sub-corneal microabscesses, folliculitis, exocytosis and cellulitis were few in these rabbits.

Cysts were often seen on the lining of the epididymal ducts, in the tunica dartos and associated adipose tissue layers, and the various layers of the small and medium sized arteries as well as veins in the testis. Occasionally, vessels appeared partially occluded due to pressure

from colonies of cysts within the walls. The pampiniform plexi of the males in this group were very heavily parasitized (Figure 20) and showed severe vascular degeneration, fibrinoid necrosis and thrombosis. Cysts were found in the walls of virtually all the branches of the spermatic artery and vein and in the loose connective tissue with a conspicuous chronic pyo-granulomatous cell reaction. Mononuclear cells, polymorphonuclear cells, multinuclear giant cells, necrotic and calcified cysts were a frequent feature of the lesions. Necrotic changes occurred in the testicular parenchyma with many of the seminiferous tubules necrotized. There was pronounced aspermatogenesis. Degenerated sertoli cells and debris were the only remaining cellular elements in most of the tubules. The tubular interstitium, the tunica albuginea and tunica vaginalis were heavily parasitized and partially necrotic. The visceral and parietal layers of the tunica vaginalis were separated by a coagulative necrotic material. Neutrophils and multinuclear giant cells predominated in the cellular reaction manifesting typical pyo-granulomatous type of interstitial orchitis, interstitial epididymitis and dermatitis. Single cysts or cyst colonies were seen in these lesions. Cysts appeared within the interstitium of muscle fibres in the tunica dartos attracting conspicuous mononuclear cell response.

In the deep skeletal muscles, no significant changes were observed except mononuclear cell infiltration and parasitization involving the muscles, tendons, synovial membranes and bone periosteum in the more distal aspects of the limbs. Cysts were found within the interstitium and the perimysial connective tissue. In the affected areas there was hyperaemia, oedema and interstitial myositis with varying degrees of angiopathy. Localized myo-degeneration and lysis were observed. A few calcified lesions with surrounding phagocytic cells in the muscles were



**Figure 20:** H/E, x100: Testicular besnoitiosis. A cyst protruding into the lumen of a vein (K) in the pampiniform plexus in a less heavily infested area.

observed in rabbit R154. Muscle fibres had undergone atrophy where cysts were located.

The larynx and trachea had no particular changes although mild congestion in the trachea was seen in one rabbit. Acute or peracute mixed cell pneumonitis associated with hyperaemia, severe oedema and haemorrhages was present in the lungs of two rabbits. Foamy macrophages occurred occasionally in the alveolar septa. Arteriosclerotic changes were observed in arteries of various sizes and in sections of alveolar walls in the lungs of rabbit R154. The same observation was made in arteries in other parts of the body. Lymphoid aggregations adjacent to respiratory bronchioles in the lungs were hyperplastic but no cyst was encountered in the lungs.

A few cysts occurred in the submucosa and the interstitium of the tongue muscles. Foci of myo-degeneration of both the transverse and longitudinal muscles of the oesophagus were seen in one case. Myo-degeneration and necrosis with calcification appeared in the inner transverse and outer longitudinal muscle layers of the ileum in two other cases. These were associated with arteritis and neuritis at the junction between the two layers.

Two degenerating *Besnoitia* cysts were encountered in the perineurium of two nerves fibres in the intestinal wall. One other cyst was seen at the center of a nerve fibre associated with wallerian type of axonal degeneration in the ear of one rabbit. Axonal degeneration and perineuritis were a frequent finding in other parasitized areas.

The eyelids, the sclera, the connective stroma of the nictitating membrane and the periorbital muscles were moderately parasitized. Mononuclear cell infiltration characterized mild keratitis and scleritis demonstrated in one rabbit. Purulent inflammation in the submucosa of the eyelid and sometimes pyo-granulomatous conjunctivitis were frequently observed. The tear glands were activated and contained masses of secretion in the acini. An artery in the dense

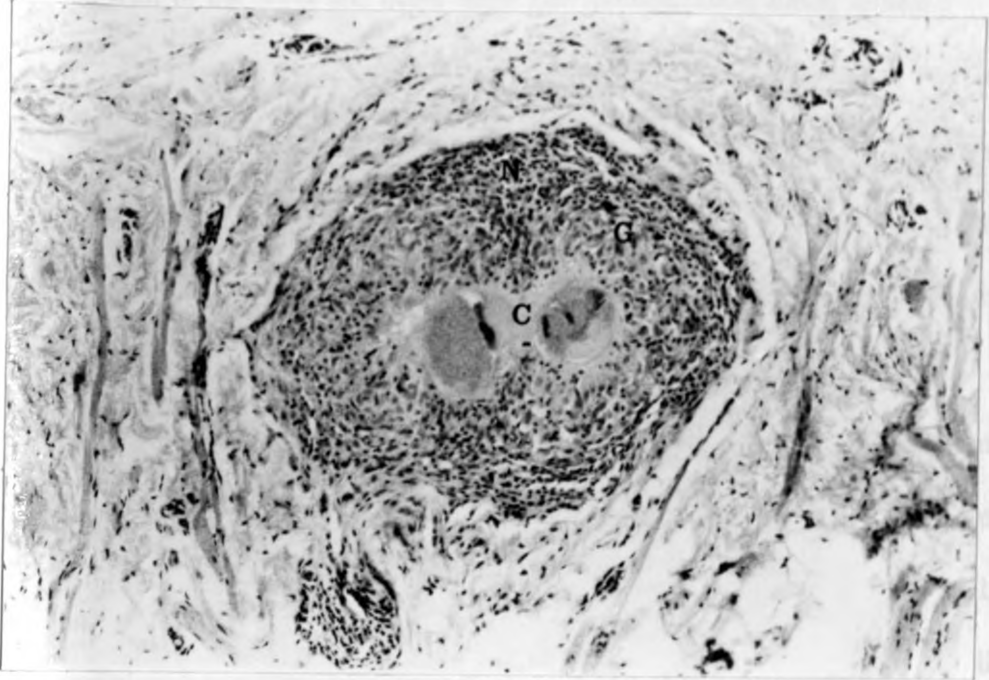
fibroelastic connective tissue of the urinary bladder was found parasitized with two cysts in R156. Mild mononuclear interstitial nephritis with perivascular cuffing and submucosal chronic cystitis were noted occasionally in the kidneys and urinary bladder respectively. These lesions were neither frequent nor uniform. The lymph nodes, the spleen, the pancreas and the liver were hyperaemic although no cysts were found in these or any other internal organs, glands and brain. An increased number of polymorphonuclear cells, especially eosinophils, and haemosiderin was present in the spleen.

#### 4.3.5. Infection of goats with caprine cyst organisms

##### Chronic stage lesions

Histopathological changes in goats infected with caprine *Besnoitia* parasites were marked by development of chronic lesions and *Besnoitia* cysts. The structural and staining characteristics of cysts were similar to those described in goats infected with bovine *Besnoitia* parasites. However, cysts were generally at a more advanced maturation stage and measured between 335 $\mu$ m to 530 $\mu$ m in diameter. The cellular reaction around the cysts was more pronounced and developed in the vicinity of live, degenerated and ruptured cysts. Lesions were primarily granulomatous with fine cellular arrangement around the cysts characterized by an inner zone of radially arranged epithelioid cells and intermingled multinuclear giant cells and an outer layer of innumerable macrophages, lymphocytes, and few plasma cells and occasional fibroblast capsule (Figure 21), a reaction that can well be described as "Besnoitia granuloma". Foci of necrotic granulomatous lesions shaped like cysts were frequently encountered without any cysts within the inflammatory reaction. Free *Besnoitia* organisms were not encountered either in the





**Figure 21:** H/E, x100: A "Besnoitia granuloma": Showing a septate caprine *Besnoitia* cyst at the middle (C), Giant cells intermingled with epithelioid cells (G), Macrophages, lymphocytes, Fibroblasts and other mononuclear cells at the periphery (N).

lumina of various blood vessels or in the lymph spaces. Moderate perivascular cell cuffing was regularly noticed in parasitized areas and was characterized by macrophages, lymphocytes, plasma cells and isolated eosinophils. Patchy degenerative lesions and vasculitis were encountered in the wall of the aorta and other large and medium sized blood vessels.

Goat GHo2 was much more severely parasitized than other goats in this group with many cysts at various stages of degeneration and calcification. Inflammatory change in this goat was marked by moderate diffuse mononuclear cell infiltration rather than localized granulomas around *Besnoitia* cysts. Granulomas were seen in the vicinity of ruptured cysts within the muscles only.

Skin sections contained many cysts in the dermis attracting intense focal reactions and perivascular cuffing. In sections of the lips and muzzle, the dermis was largely replaced by enlarged cysts in colonies only separated by their thick fibrous capsules. Associated blood vessels were heavily parasitized and contained cysts across the various layers of the wall and adventitia. *Besnoitia* cysts with surrounding granulomas were often encountered growing towards the lumina of the vessels (Figure 22). Tactile hair follicles were generally less parasitized in this group. The epithelium of the skin was either normal or atrophic where superficially placed mature cysts were found. A few denuded sections of the mucosa were also noticed. Similar extensive parasitization was found in the tail and the teats. Many cysts were found underlying the epithelium of the teat canal (Figure 23) and causing pressure atrophy of the tissues of the teat. Acanthosis, parakeratosis, mild oedema and diffuse mononuclear cell infiltration were more common in the skin of the ears and eyelids. Purulent inflammation in submucosa, exocytosis and sub-corneal microabscesses were also frequently encountered. Subacute conjunctivitis.

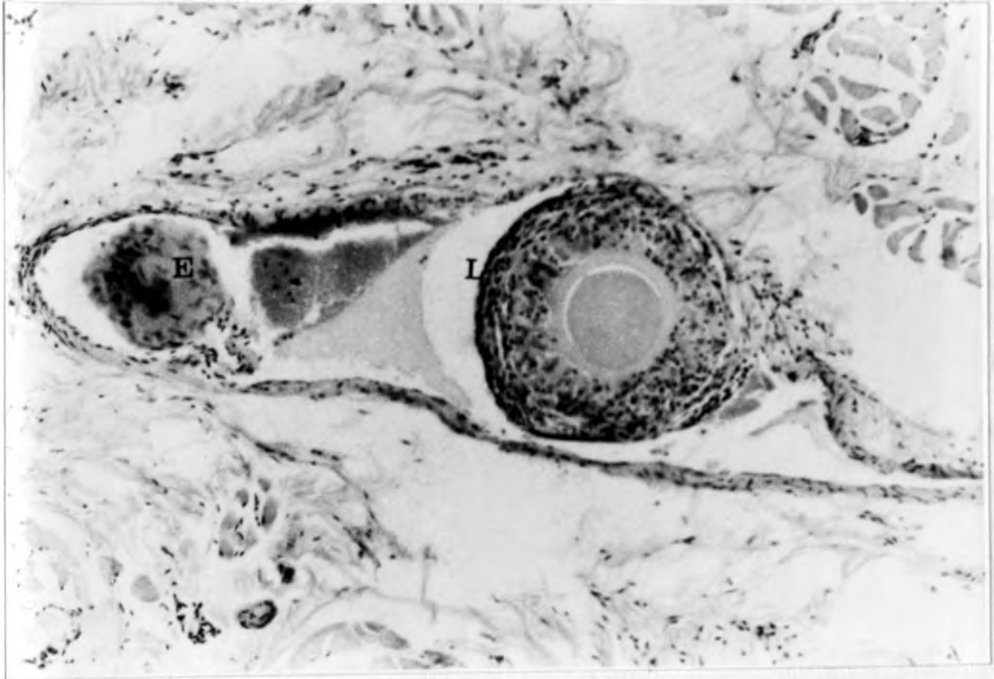
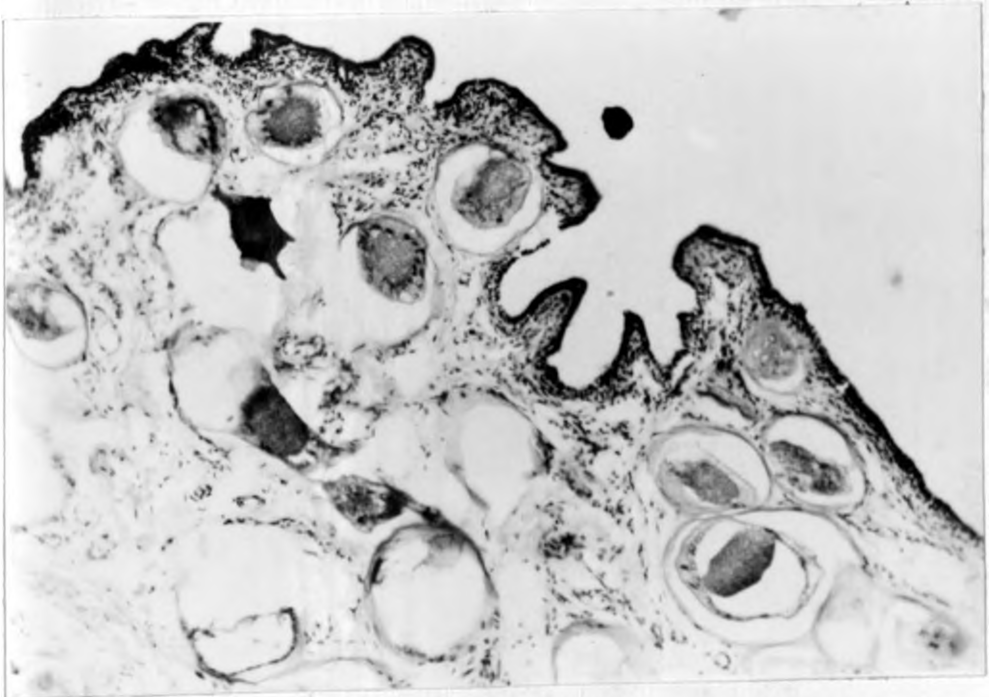


Figure 22: H/E, x100: A "Besnoitia granuloma" protruding into the lumen of a blood vein (L) and a degenerated *Besnoitia* granuloma embolus(E).

Figure 23: H/E, x100: Masses of *Besnoitia* cysts in a teat canal of a goat infected with caprine *Besnoitia* cyst organisms. The cysts have occupied and replaced the teat tissues and mucosal erosion was evident in some areas.

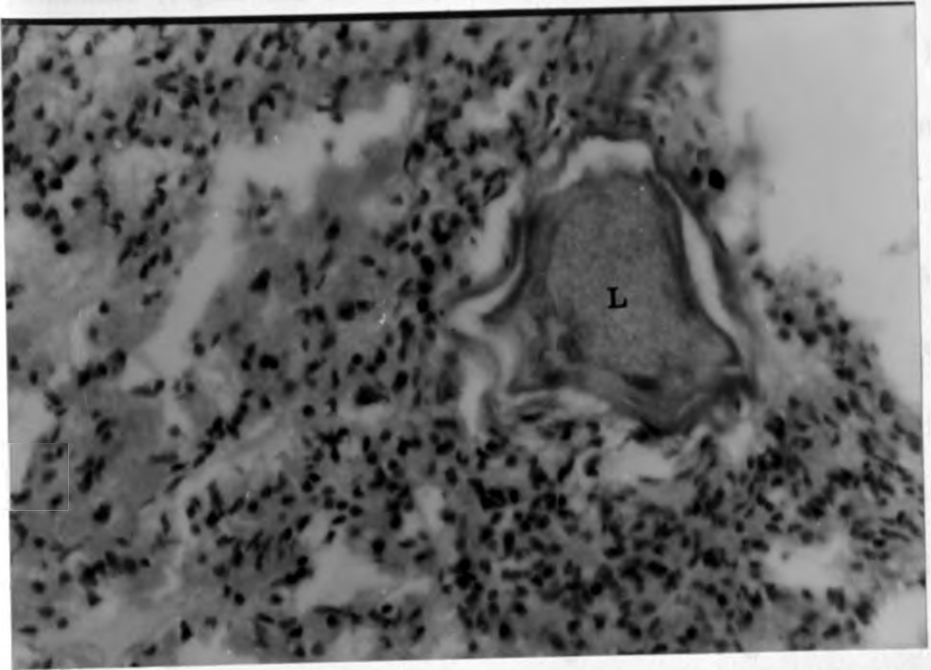


hyperplastic lymphoid aggregations and mucosal denudations were commonly observed in the eyelids.

Periorbital muscles in the eyes, superficial skeletal muscles, and the tendons and other fibrous tissues of the limbs were either moderately or heavily parasitized with *Besnoitia* cysts. The cellular host response was as severe in these sections as it was in other parasitized areas. Intense granulomatous response was elicited by apparently live, degenerated or ruptured cysts in the skeletal muscles. Foci of myo-degeneration or myositis were found around severely parasitized sites. Synovial sheaths, tendons and periosteal connective tissue contained *Besnoitia* cysts. Frequently, the continuity of small blood vessels was apparently interrupted by the development of cysts.

The stroma of the nasal septum mucosa and the turbinates contained numerous cysts. Blood vessels and the lymphatics in these locations were extensively parasitized. Associated bundles of skeletal muscles, cartilage and skin adnexa had undergone atrophy and numerous colonies of cysts took their place. Degenerative changes and cellular infiltration were encountered in the remaining glandular and muscular tissues. Submucosal mononuclear cell infiltration was noted in the larynx and trachea. Atelectasis, hyperaemia and mild oedema were encountered in the lungs and in one case two degenerating cysts were present in the alveolar septae (Figure 24). The lymphoid tissue was moderately hyperplastic.

*Besnoitia* cysts were found in the lymph nodes, spleen, kidneys, rumen, hypophysis, vascular plexus of the circle of Willis and related nerve ganglia. A significant number of cysts were encountered within the medulla, the cortex and in the subcapsular area of the submandibular and pre-scapular lymph nodes. Cysts in the cortex were surrounded by lymphoid

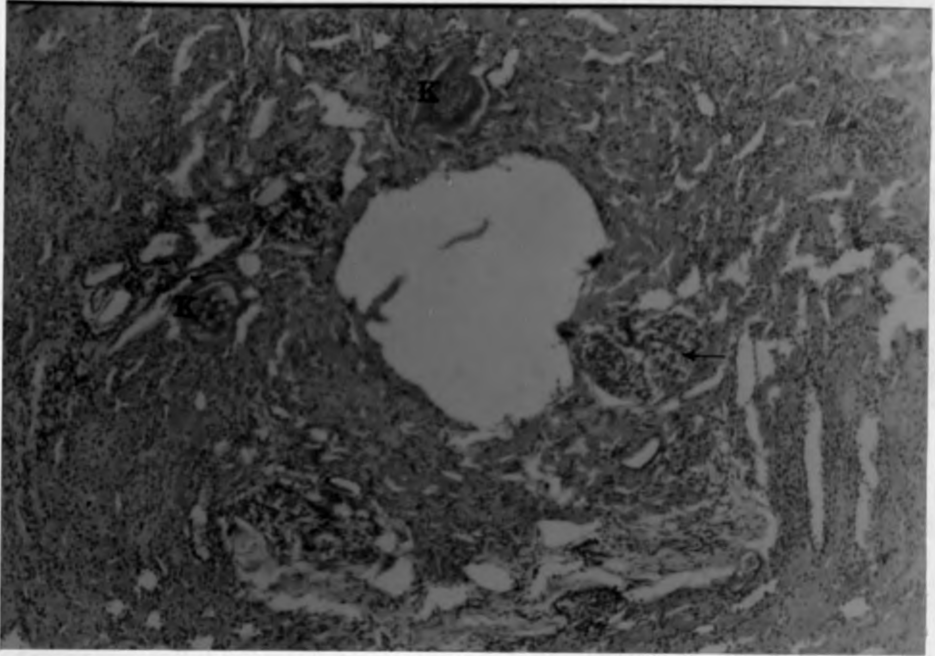


**Figure 24:** H/E, x400: A degenerated *Besnoitia* cyst (L) in the alveolar septa of the lung.

cells and did not appear to be associated with the trabeculae. Hyperaemia, prominent hyperplasia with very active germinal follicles and dense cellular medullary cords were seen in the lymph nodes. In two cases a few intact looking cysts were noticed in the kidneys (Figure 25). Foci of interstitial nephritis dominated by lymphocytes and macrophages were also seen. In GHo2, the adenohypophysis, related nerve ganglia and the blood vessels around the circle of Willis plexus were severely parasitized (Figure 26a and 26b) and attracted moderate mononuclear cell response. Moderate lymphocytic infiltration in the adventitia of the vessels of the plexi in the other two cases was encountered. Mild or severe mononuclear cell reactions were obtained in the submucosa of the tongue. Extensive epithelial necrosis and ulceration was encountered in the rumen of GHo2 but no cysts or *Besnoitia* related lesions were encountered in this organ after subsequent sections were examined. Staining with PAS ruled out fungal infection in this lesion. The liver showed occurrence of irregular foci of mononuclear cell infiltration around the portal triads in one case. Closer observation revealed close association of the lesions with the bile ducts. No cysts were encountered in the liver. Sections of the urinary bladder, the uterus and elsewhere in the female reproductive tract revealed no significant changes. Sections of the adrenals, myocardium, pancreas, oesophagus, reticulum, intestines, the brain and meninges were also free from cysts or related lesions.

#### 4.3.6. Infection of rabbits with caprine cyst organisms

Rabbits infected with the caprine strain developed occasional mild mononuclear cell infiltration around the portal triads in the liver, in the myocardium and the kidneys. Mild mononuclear cell dermatitis associated with mild perivascular cell cuffing, acanthosis and



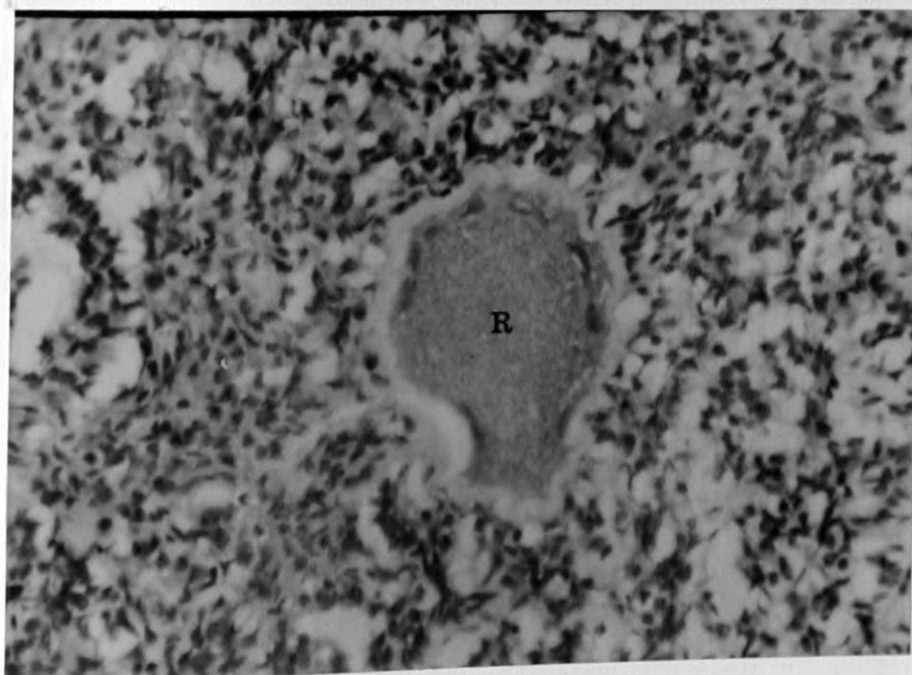
**Figure 25:** H/E, x40: *Besnoitia* cysts (K) in the kidney from a goat infected with caprine *Besnoitia* cyst organisms. (→) shows a glomerulus. The cysts were located within the interstitium.





Figure 26a: H/E, x40: *Besnoitia* cysts within the veins of the circle of Willis (W) of a goat infected with caprine *Besnoitia* cyst organisms. The adenohipophysis (A) was also infected (Figure 26b).

Figure 26b: H/E, x400: *Besnoitia* cyst (R) within the adenohipophysis shown in figure 26a.





folliculitis was encountered in the skin of the ears in one rabbit. Localized heavy mononuclear cell infiltration and partial fibrinoid degeneration in a few small arteries was noted in another.

The scrotum, testis, epididymis and the pampiniform plexus in the male rabbits were apparently free of *Besnoitia* cysts or any inflammatory changes. The seminiferous tubules and the epididymis were active and contained masses of spermatozoa. The tongue, the lung, the heart and the trachea were mildly hyperaemic. No other lesions or organisms were detected in the muscles, the body skin and the skin around favourite areas of the head, the tail, the eyes or in the internal organs, glands or the brain. The lymph nodes and the spleen in the rabbits had no notable reactive changes.

#### 4.3.7. Controls

No clinical signs were noticed in a group of rabbits and goats kept as control animals. These animals survived the entire study period in good body condition. Blood and lymph node smears revealed no parasites. Leucocyte cell counts of the control goats increased in numbers between the fourth and the fifth week of the experiment (Figure 3). Packed Cell Volume (PCV) values were consistently within the normal ranges (Schalm *et al.*, 1975).

Post-mortem and histopathological examination of goats, Gc1 and Gc2; and rabbits R126 and R128 was done. These goats and rabbits were in good nutritional state and there were no gross lesions seen. Histopathological examination of the cutaneous, vascular and muscular tissues and internal organs revealed no significant findings.

## DISCUSSION

This study sought to determine whether *Besnoitia* occurring naturally in goats was related to *B. besnoiti* of cattle. Bwangamoi *et al.* (1989) speculated on the existence of a strain of *Besnoitia* specific to goats. Since then atypical natural infections have been encountered in goats in Kenya (Bwangamoi, 1989b). Parasites of the genus *Besnoitia* are very similar morphologically. This was also true for the caprine and bovine isolates used in this study were no exception. Contrary to expectation, two sets of results were obtained from the two parasites.

Bovine isolates were highly pathogenic to both rabbits and goats. Acute and often fatal reactions followed infection in both rabbits and goats. Typical syndromes of besnoitiosis as described by Basson *et al.* (1970) and Pols (1960) were reproduced in both host species after infection with the bovine isolates. The temperature reactions were pronounced and sustained in rabbits and goats. The febrile period was reduced by three days in recipients of proliferative blood parasites. Typical generalized oedema occurred in all hosts infected with bovine isolates. Perhaps this was a manifestation of lack of adaptation of the bovine *Besnoitia* parasites to goats and rabbits whereas it is highly adapted to its natural host. It is considered that methods of infection used in this study are methods which could occur in nature. What constitutes an infective dose in natural *Besnoitia* infections is unknown. While disruption of blood vessels by parasites and cysts leads to oedema amongst other vascular lesions; a parasitic toxic factor secreted by the bovine strain may be involved in the severe oedema prevalent in the acute stage and the resulting extreme emaciation and debility in infected goats and rabbits. Vasculitis and fibrinoid degeneration involving arterioles and veins without cysts

was a common feature. Karstad *et al.* (1983) have associated arteritis in flamingoes with *Besnoitia* - like parasites. A similar association cannot be ruled out in our study. Secondary complications occurred as conjunctivitis and acute respiratory distress. The chronic dermatitis characteristic of chronic besnoitiosis was not demonstrable in the goats. However, skin lesions in the rabbits were severe and showed tendency to progress to chronic dermatitis similar to that described in the bovine (Hofmeyer, 1945). Painful testicular swellings occurred in the males. The testicles were turgid and hot. Sections of the skin in the cooler parts of the body, ears, muzzle, testicles and the distal parts of the limbs in the rabbit became gangrenous and necrotized. *Besnoitia* parasites were encountered in the serosanguinous discharge and the necrotic material.

In contrast to results shown by the bovine *Besnoitia* strain, the caprine strain of *Besnoitia* behaved differently in rabbits and goats. This *Besnoitia* spp. elicited a mild clinical syndrome in goats marked by a pronounced but short - lived febrile reaction. One goat in this group developed mild and transient oedema swellings and respiratory distress due to a ruminal ulcer unrelated to besnoitiosis. The ulcer perforated and caused death. The typical anasarca syndrome caused by infection with the bovine isolates was not demonstrable, while the goats remained in good body condition throughout the study. Chronic besnoitiosis was the typical disease and *Besnoitia* cysts were clinically observable on the conjunctiva of these goats 2 - 3 weeks after the febrile phase. An extensive development of *Besnoitia* cysts and lesions encountered at necropsy and histopathological examination unmatched the mild clinical course.

It is notable that eight rabbits inoculated with bovine cyst organisms died within 24 hours. This was irrespective of the route of administration and the dosage. Respiratory distress

expressed before death in one rabbit and the vascular lesions encountered after death are signs of circulatory failure. Evidence of toxicity by cyst forms of *B. besnoiti* in rabbits has been reported (Besnoit and Robin, 1912; Bigalke, 1967). Besnoit and Robin (1912) found guinea pigs and rats refractory to the toxin. Toxins of coccidian protozoa have been documented in *Sarcocystis* (sarcocystin), *Eimeria* (Eimeria toxin) and *Toxoplasma* (toxotoxin) (Lunde and Jacobs, 1964; Tonjum, 1962; Woodworth and Weinman, 1960). Rabbits have previously been found suitable specimens for studies in bovine besnoitiosis (Bigalke, 1960; Pols, 1954a, 1954b, 1960). The deaths demonstrated in this study may be attributed to high toxicity or high rate of proliferation that may be associated with the bovine *Besnoitia* strain isolated in Kenya. The caprine isolate used in the present study perhaps did not produce any signs of toxicity in either host species. May be different *Besnoitia* strains produce variable amounts of toxin. Further studies on the existence of *Besnoitia* toxin (bovine strain) will be necessary before conclusions can be made. A mild clinical reaction followed infection with the caprine isolate in rabbits. A transient temperature reaction ensued after a prolonged incubation of 11 days. The rabbits also maintained a good nutritional status throughout the study.

Although proliferative parasites were only occasionally seen in blood, their presence in blood of goats infected with bovine *Besnoitia* strain was proved when their blood, collected at the peak of pyrexia, was sub-inoculated into rabbits and goats to reproduce the characteristic *Besnoitia* syndrome. Only one report of artificial transmission of besnoitiosis from chronically infected cattle to goats has been previously reported (Pols, 1960). One goat out of four infected with bovine strain cyst organisms reacted severely. Close details of the abnormal cytoplasmic extensions in monocytes encountered in this study were not obtained. However, in this study,

monocytes are thought to be the earliest cells to be parasitized by *Besnoitia* and may therefore be largely responsible for dispersal of the parasite in the body. Pils (1960) demonstrated *Besnoitia* parasites in monocyte cytoplasmic extensions further strengthening the role of monocytes in spreading the parasite in the body of an infected animal. The parasites were not arranged in any organized manner within the cells.

Proliferative organisms were also absent from blood smears of rabbits and goats infected with caprine *Besnoitia* isolates. The low parasitaemia of *Besnoitia* organisms in blood of infected animals has been documented (Bigalke, 1967). Although less pronounced, the monocytosis was found to be of value in indicating some degree of response in this group and it may account for the survival of goats and rabbits infected with caprine *Besnoitia* organisms through the study period. Whether the proliferation of monocytes in *Besnoitia* infections is only part of a cellular immune response or is initiated by other specific parasitic factors is unknown. The thermal and mild leucocytic reactions by the rabbits was puzzling and needs further investigation. However, there is scientific evidence to suggest that rabbits are on the whole refractory to caprine isolates of *Besnoitia* such as those used in this study (Njenga *et al.*, 1993). More work need to be done however to evaluate the virulence and pathogenicity of the caprine strain when transmitted to cattle and rabbits in cyst and proliferative forms.

Total leucocytic cell counts in the three study groups of goats produced diphasic response curves. Single peaks were obtained in rabbits infected with either strains. The initial peaks coincided with the febrile reactions while the second coincided with the acute clinical phase after termination of fever. The rise and fall was more gradual and the peaks were sustained longer in goats and rabbits infected with the caprine isolate. This may be a further support to the

importance of development of a cellular immune response in survival of animals infected with *Besnoitia* (Frenkel, 1967; Frenkel and Lunde, 1966; Frenkel and Wilson, 1972; Hoff and Frenkel, 1974). The role of the immune response in the pathogenesis or in inducing cyst formation in *Besnoitia* infection needs further studies. The described peaks may be artefacts arising from the fact that some animals died within the study period and therefore their leucocytic counts did not cover the entire study period. However, their consistency across the three study groups is remarkable. Random selection and sizes of study groups led to differences in WBC counts pre-infection but these were all within the normal ranges for the host species. Pathologists have previously described besnoitiosis in three clinical stages (Bwangamoi, 1979; Soulsby, 1982). Perhaps the leucocytic cyto-dynamics of besnoitiosis would only serve to enhance such phasic descriptions and may manifest immune reactions to the initial intravascular parasite multiplication and the latter extravascular proliferation. The peak obtained in the control curve between the fourth and the fifth weeks could not be explained and was not related to any other clinical observations. Whereas a steady rise in leucocytic cell counts was maintained following infection of rabbits with bovine blood parasites, the peak was sharply broken into an irregular pattern of values close to control values following infection with the caprine isolate.

Packed cell volume (PCV) values in all test groups were consistently below control values. Pre-infection differences in PCV values were also attributed to the random selection and sizes of study groups but they also fell within normal ranges for the host species. Goats and rabbits infected with bovine isolates, in either form, were more stressed and gave comparatively lower values. The PCV curve for goats infected with bovine cyst organisms showed a sharp peak between the second and third week post-infection which could not be explained.

Histopathological studies confirmed the clinical observation of high virulence of the bovine isolate. The primary lesion was evidently vascular in nature. Haemorrhages, vasculitis and fibrinoid necrosis of arterioles and venules were the predominant lesions. These were often accompanied with hyperplasia and hypertrophy of various vascular and perivascular cells. Capillaries were often occluded with dislodged endothelial cells, microthrombi or large multinucleated cells in affected tissues. Emboli of cysts detached from the endothelial lining or ruptured cysts were encountered in the lumen of some blood vessels and serous spaces in the joints. Basson *et al.* (1970) documented parasitization of endothelial cells and the primitive mesenchymal cells of the vascular intima. The possibility that cysts rupture periodically, releasing their contents into the circulation has been postulated (Besnoit and Robin, 1912; Bigalke, 1967 and McCully *et al.*, 1966). Numerous *Besnoitia* cyst emboli observed in this study further confirm these postulates. In addition, *Besnoitia* cysts protruding and almost occluding blood passages were a common feature. These could eventually break into circulation as emboli or bradyzoites to be disseminated into other locations. Thrombosis and vascular occlusion result in ischaemia, infarction and tissue necrosis.

Intense vascular damage coupled with the accompanying inflammation and parasitic proliferation interfered with blood supply causing degenerative changes in affected areas. Acanthosis, parakeratosis, scleroderma, panniculitis, focal dermatitis, superficial myodegeneration, myositis and lysis of muscle fibres were common in both host species under study. Some skin areas on the rabbits necrotized altogether. Vascular lesions often occurred in organs that did not develop *Besnoitia* cysts. Myocardial degeneration occurred in both hosts species. In addition, arteriosclerosis was demonstrated in the heart, aorta, pulmonary artery and several

her smaller peripheral arteries in the rabbits. These were not necessarily associated with proliferation of parasites although vascular degenerative changes can enhance calcium deposition in these vessels. No cysts or inflammatory reaction was encountered in the heart of any of the hosts. Myo-degeneration and calcification was also noted in the intestinal tract of one rabbit. This was associated with arteritis and neuritis in the nerve plexi. Such secondary pathological changes associated with the bovine strain of *Besnoitia* have been widely reported before (Basson *et al.*, 1970; Bigalke, 1967; Bigalke *et al.*, 1967). Such degenerative changes have been attributed to toxins elaborated by the bovine *Besnoitia* strain or may result from immunological reactions to circulating parasite antigens. Such lesions were rarely demonstrated in goats and were completely absent in the rabbit recipients of the caprine *Besnoitia* strain.

The cellular reaction in animals infected with bovine *Besnoitia* isolates was chiefly mononuclear with a slight sprinkling of neutrophils. The most characteristic cellular infiltration occurred in the cutaneous and superficial muscular tissues. Cheema and Toofanian (1979) reported similar characteristic cellular reactions in muscular tissue. Whether a manifestation of tissue specificity exists in besnoitiosis, it will require further investigations. Eosinophils were encountered only in the sparse foci of myositis, mixed cell pneumonitis, folliculitis and subcutaneous panniculitis in the scrotum of the male rabbits. This sharply contrasts the findings of Basson *et al.* (1970) who found a consistent eosinophilic reaction. The eosinophilic reaction exhibited allergic type of reaction in particular tissues that will require further elucidation.



The presence of parasites in the tissues was associated with proliferation of histiocytes. However, not all histiocytes were shown to be parasitized. Clusters of degenerative histiocytic cells were a common feature.

In goats infected with the bovine strain of *Besnoitia*, inflammatory cells were not attracted by the cysts, especially where cysts developed strictly within the connective tissue. In fact, degenerative cysts often occurred in absence of an accompanying host response. However, occasional cysts located deep within the superficial muscle fibres and one situated in the media of a hepatic vein aroused moderate mononuclear cell response. Severe host response to cysts in the skeletal muscles with little response occurring in other areas has been reported in antelopes (McCully *et al.*, 1966). This was in contrast to the pronounced and consistent granulomatous reactions that accompanied cysts in rabbits infected with the bovine strain of *Besnoitia*. Necrotized and calcified cysts also attracted intense cellular reactions. It may appear that the initiation of chronic granulomatous reactions is an inherent property of the parasite in preferred hosts such as the rabbit.

The cellular reaction around cysts in goats infected with caprine *Besnoitia* isolates was more pronounced than in goats infected with the bovine isolate. The lesions obtained in the former group were typically granulomatous with uniform cellular arrangement into "Besnoitia granulomas". These granulomas had an inner zone of radially arranged epithelioid cells intermingled with multinucleated giant cells, surrounded by an outer layer of many macrophages, lymphocytes, few plasma cells and occasionally by a connective tissue capsule. Similar findings have been reported by McCully *et al.* (1966). In the same goats some granulomas were encountered without any cysts in the vicinity. It was assumed that either the cysts had been

completely digested in the inflammatory process or the lesions were tangential cuts during sectioning. However, such lesions were few and far between. Interestingly, one goat, GHo2, showed a severe and more diffuse mononuclear cell reaction with less organized granulomas around the cysts. Cellular reaction was encountered around ruptured, degenerative as well as live cysts. This contradicts the theory that the inflammatory reaction does not occur around intact cysts (Schulz, 1960). It is argued that since cysts are inside mononuclear phagocytes they are not attacked by inflammatory cells until they rupture (Bwangamoi, 1989b). Basson *et al.* (1970) and McCully *et al.* (1966) constantly found mononuclear and granulomatous reactions around many cysts in which no degenerative or necrotic changes could be detected by light microscopy. Release of parasite soluble antigens or diffusion of other soluble factors out of the cysts could attract inflammatory cells. Perhaps the entirety of such cysts will require more tests such as electron microscopy to verify.

Systematic investigations on the differential distribution of cysts showed a special affinity for areolar connective tissue of the dermis and subcutis, mucous membranes, sclera, male genital tract, superficial muscles, superficial vascular system, bone periosteum and cartilage perichondrium. This demonstrated a preference to parasitize the cooler and more superficial parts of the body. The areolar connective tissue of the perineurial tissues and the dense connective tissue of the joint capsules was affected to a lesser extent after infection with bovine *Besnoitia* isolates. Dense concentration of cysts occurred in the nose, nasal septum, lips, muzzle, ears, tail, testicular connective tissue and the pampiniform plexi. It has been noted before that the high concentration of skin cysts in certain sites may have a bearing on transmission since

sects are known to have preferential feeding sites (Bigalke, 1968). Findings in this study further strengthen the above hypothesis by Bigalke (1968).

In addition, the teat canal and the dermis of the teats were densely parasitized in goats infected with caprine *Besnoitia* isolates. Epidermal and mucosal necrosis, ulceration and excoriations were encountered in such severely parasitized areas. The significance of such excoriations and lesions in transmission of besnoitiosis is a high possibility. Epidermal and nasal secretions and secretions could provide suitable areas for collection of parasites by arthropod vectors in the process of feeding. Moreover, infection of goat-kids by suckling the parasites from the surface of the teat as well as in the milk should also be considered. Bigalke (1968) has experimentally infected cattle and rabbits orally with trophozoites. It is therefore possible that goat-kids can also be infected orally when suckling their infected dams, especially if teats are heavily infected as observed in this study. Furthermore, *Besnoitia* cysts have been found in histological sections of skin biopsy from the ear of a 20-day-old goat-kid (Bwangamoi, 1989b). Secondary dermatitis and conjunctivitis occurred where the integrity of the cutaneous or serous surfaces was interfered with by presence of cysts or inflammation. Colonies of bacteria and microabscesses were regular findings.

Hair follicles and other cutaneous adnexa were not directly involved in parasitic proliferation after infection with caprine *Besnoitia* isolates. However, degenerative and atrophic changes were seen where large colonies of cysts occurred. Vascular and perivascular lesions in other tissues were also less extensive in recipients of this strain than those obtained in goats infected with the bovine isolate. The latter group of goats were however sacrificed at the chronic stage, a time when such lesions may have resolved. The severity of the clinical syndrome

appears to be closely linked to the extent of the vascular lesions as observed in caprine and rabbit recipients of bovine *Besnoitia* strains. Basson *et al.* (1970) reported similar observations in experimentally infected cattle and rabbits. Indeed, the present studies indicate that cardiovascular pathological changes produced in infections with caprine strains in the natural hosts are subclinical as opposed to acute and subacute disease in goats infected with *B. besnoiti*.

Parasitization of the ocular structures, eyelids, sclera, nictitating membranes and periorbital muscles was moderate in animals infected with bovine *Besnoitia* strain. In addition, *Besnoitia* cysts were encountered in the sweat glands and hair follicles in the rabbits, but not in the goats, where they attracted intense mononuclear cell reactions. Mild perineuritis and axonal degeneration of peripheral nerve fibres was seen in a few affected tissues. Three cysts encountered in nerve fibres in the rabbits appeared degenerated. It was not clear what cells were parasitized in these areas although it was most likely histiocytic cells because of their migratory characteristic. One cyst was encountered in a sub-mandibular lymph node in one goat. Clusters of histiocytic cells were also within the lymphoid follicles. It was difficult to establish whether they were parasitized or not. Two other cysts encountered within the adventitia of the urinary bladder were located in the intima of an artery. This was an unusual location for *Besnoitia* cysts.

The deep skeletal muscles, gastrointestinal tract, central nervous system, kidney, the female reproductive tract and the cutaneous and mucosal epithelium in animals infected with bovine *Besnoitia* strain had no *Besnoitia* cysts. Tangential sections of cysts within the rete pegs of the dermis often made it appear as if *Besnoitia* cysts occurred within the epidermis. Cysts encountered in the distal muscles of the limbs in rabbits aroused intense host response, atrophy and lysis of muscle fibres.

Although most internal organs in recipients of bovine *Besnoitia* organisms were largely free of *Besnoitia* cysts and the characteristic inflammation, acute interstitial pneumonia was a common secondary complication. Degenerative changes of the renal tubules and hepatic cells were common. The changes consisted mainly of cloudy swelling in the renal tubules and vacuolar and hyaline droplet degeneration in hepatocytes. Mild foci of interstitial nephritis and cystitis were seen in one rabbit and their source could not be elucidated. No *Besnoitia* cyst was encountered in the adrenal glands in any of the experimental hosts. This is in contrast to observations by Frenkel (1977a) and Smith and Frenkel (1977) who reported adrenal glands as preferred sites of parasitization.

Evidence of invasion of unusual sites and deep-seated internal organs was obtained in goats infected with the caprine *Besnoitia* spp. *Besnoitia* cysts are not known to occur in such deep-seated organs. *Besnoitia* cysts were seen in the alveolar septae in the lungs, kidneys, liver and ruminal muscles. Cysts encountered in the lungs were degenerative, whereas those in the kidneys appeared intact and associated with foci of interstitial nephritis. In the respiratory tract the highest concentration of cysts is expected in the anterior part, up to the bifurcation of the bronchi. The number of cysts is drastically reduced further down, so that in the parenchyma only a few isolated and degenerated cysts are found (Bwangamoi, 1989b; Kaliner, 1973). Mbutia *et al.* (1993) have however demonstrated intact undegenerated cysts in lungs of a rabbit. The spleen, the sub-mandibular and pre-scapular lymph nodes contained a significant number of *Besnoitia* cysts. The cysts in the lymph nodes were not confined to any particular histological zone. Parasitized and non-parasitized lymph nodes showed a marked proliferation of lymphoid cells. This could be a sign of increased activity in the cellular immune response. That cell-

mediated immune reactions are elicited in response to *Besnoitia* infection has been confirmed (Frenkel, 1967; Frenkel and Lunde, 1966; Frenkel and Wilson, 1972; Hoff and Frenkel, 1974). Parasitization of the adenohypophysis, related nerve ganglia and the vascular plexus of the circle of Willis was a particularly striking feature in one goat infected with the caprine isolate. These changes were not seen in the other goats of the same group. A ring of perivascular lymphocytic infiltration around the cysts was a constant appearance in these plexi. Except in the present study, *Besnoitia* cysts have not been reported in the tissues of the central nervous system in any host before although cysts can occur in almost any tissue containing blood vessels (Bwangamoi, 1979). Wide surveys will be required to establish the exact distribution of *Besnoitia* cysts in natural caprine besnoitiosis. It may be that caprine strains of *Besnoitia* have different predilection sites. Such differences have been applied before to differentiate antelope strains of *Besnoitia* from those of cattle (Basson *et al.*, 1965; Basson *et al.*, 1970; Bigalke *et al.*, 1967). While infrequent clusters of hypertrophied histiocytic cells were encountered within some internal organs, the identity of other parasitized extravascular and perineurial cells could not be established with any certainty. Viscerotropic strains of *B. besnoiti* are documented in the cardiovascular system of antelope (McCully *et al.*, 1966), in goat lungs (Kaliner, 1973; Cheema and Toofanian, 1979) and rabbit lungs (Mbuthia *et al.*, 1993).

Lesions in the male reproductive organs were characteristic in both host species after infection with bovine *Besnoitia* organisms. They were primarily vascular and included oedema and necrosis. The histological architecture in the testicles was often severely damaged and blood supply disrupted by proliferation of cysts and the accompanying host reaction. Degenerated

Spermatid and Sertoli cells were often the only remaining cellular elements. These lesions could affect the fertility of the animals permanently. Evidence to support this observation has been presented previously (Kumi-Diaka *et al.*, 1981; Pols, 1960; Schulz, 1960). Bwangamoi (1989b) attributes infertility in affected bucks to the presence of masses of *Besnoitia* cysts in the pampiniform plexus. This seriously reduces the supply of cool blood to the testis, necessary for spermatogenesis. Toxins from the cysts have also been cited (Cheema and Toofanian, 1979). It may therefore be that a toxin is responsible for the extent of degenerative changes in the parenchymatous organs, heart muscles and blood vessels (Basson *et al.*, 1965; 1970). Further investigations are necessary to determine the real cause of these degenerative changes in these parenchymatous organs. The healthy status of breeding in female hosts is attributed to the fact that the ovary, like other deep seated organs, is unfavourable for the growth of *Besnoitia* cysts. It may be interesting to make a more comprehensive study of this aspect in caprine besnoitiosis where a significant incident of cysts in visceral organs was noted. Ernst *et al.* (1968) has reported cysts of *Besnoitia jellisoni* in reproductive organs of rodents while Nobel *et al.* (1977, 1981) have encountered *Besnoitia* cysts in the vagina and uterus of a cow. In this study the ovary and the entire reproductive tract in five does infected with the caprine isolate were, however, free of cysts. Involvement of a hormonal refractory factor cannot therefore be ruled out. These does were not mated during the experiment. It is possible that cysts reported by these authors occurred through coitus or different strains of *Besnoitia* may have different predilection sites in a host. The caprine *Besnoitia* strain here may not be predilected to female reproductive organs.

Bwangamoi (1989b) reported morphological differences between mature *Besnoitia* cysts from the testicles of a goat and mature cysts from other organs. There were no males in the study group of goats infected with caprine *Besnoitia* organisms and therefore comparative testicular lesions were not obtained. No morphological differences were encountered between testicular cysts and cysts from other organs in caprine and rabbit bucks infected with bovine *Besnoitia* isolates. Such specific comparisons would require consideration of a large number of cysts preferably sectioned at the same plane if they were to be statistically significant.

The primary lesions of vasculitis, vascular degeneration or necrosis involving either the intima or the entire walls of the veins and arteries, thrombosis and vascular parasitization (Basson *et al.*, 1970) were virtually absent in the commonest localities in rabbits infected with caprine *Besnoitia* isolates. Typical skin and testicular lesions were also absent in these rabbits. A systemic necropsy and histopathological examination of the skin and other tissues showed no *Besnoitia* cysts 80 days post-infection. This contrasted with the severe lesions and *Besnoitia* cysts encountered in similar localities in rabbits infected with the bovine isolate. A few small foci of nononuclear cell infiltration encountered in the liver and kidney of the rabbits infected with the caprine *Besnoitia* parasites were inconsistent and could only be associated with spurious underlying focal reactions. Their close association with bile ducts in the liver was perhaps evidence of secondary cholangitis. Similar lesions were not encountered in any other localities in these rabbits and were only mildly present in the goats. Secondary dermal reactions were largely absent in the rabbits. Masses of spermatozoa were encountered in the spermatic ducts in the males, a clear evidence of spermatogenesis.



It is significant that the caprine isolate was only mildly pathogenic to rabbits. Absence of testicular and skin lesions in rabbits infected with the caprine isolate was in contrast to the severity shown by infection with the bovine isolate. The strain produced sub-clinical infection in goats unmatched by the extensive development of chronic cysts. Goats infected with either cyst or proliferative parasites of the bovine isolate rarely survived the acute phase. The disease terminated fatally in all but one goat and one rabbit. No particular cyst characteristic or distribution differences were noted between infection with cyst and proliferative bovine organisms. However, cysts were more common in the interstitium of the muscles and blood vessels in the rabbits than in the goats. Sweat glands, ducts and large arteries of the tactile hair follicles amongst the cutaneous adnexa were additionally parasitized. Moreover, the appearance of more differentiated cysts in rabbits could mean that the parasite proliferates more rapidly in this host. The suitability of rabbits as hosts for *B. besnoiti* is documented (Pols, 1960). Whether they are equally suitable for the Kenyan caprine *Besnoitia* strain, especially in its primary isolation will need further studies.

The fact that acute reactions were not observed in either the goats or the rabbits may suggest a low pathogenicity of the caprine strain, in reference to the clinical manifestations and the pathology of experimental infections. However, the present study does not suggest that the proliferative parasites of the caprine strain would produce similar results. Indeed, differences in pathogenicity of the two stages have been expressed in other studies of *Besnoitia* strains during passage (Basson *et al.*, 1970). Differences of pathological changes were sometimes noticed. Although bovine *Besnoitia* strain has been shown to produce similar lesions in cattle and

rabbits (Pols, 1960), Basson *et al.* (1970) demonstrated more severe vascular lesions and less development of *Besnoitia* cysts in rabbits infected with high passage-level bovine strain than in cattle infected with recently isolated strains. Where differences of pathogenicity to a given host species are marked following infection with primary isolations of a parasite, it can only be attributed to differences in virulence of the strains. Adaptation to a given host may however differ with different strains of protozoa (Dubey, 1977). Virulence to susceptible natural and laboratory host species has previously been applied to differentiate bovine and antelope strains of *Besnoitia* (Basson *et al.*, 1970; Bigalke *et al.*, 1967). Strain differences based on virulence for natural and laboratory hosts are documented in *Toxoplasma*, a coccidian protozoa closely related to *Besnoitia* (Dubey, 1977). Since biological differences are expressions of genetically endowed metabolic differences between strains, the use of virulence to both natural and laboratory hosts as a taxonomic tool is valid. Experimental evidence at this stage is scanty but would appear inappropriate to regard goats infected with the caprine strain as reservoirs of bovine besnoitiosis as we know it today.

Although factors governing the pathogenicity of *Besnoitia* are yet to be elucidated, the findings in the present study clearly illustrate biological differences between the bovine and caprine *Besnoitia* strains. Whereas the bovine isolate was highly pathogenic to both goats and rabbits and induced an acute and often fatal anasarctous syndrome, the caprine *Besnoitia* strain was only mildly pathogenic to both host species although it produced abundant *Besnoitia* cysts in the goats. The latter cysts elicited a peculiar and consistent granulomatous reaction in the goats. Moreover, the caprine strain was more inclined to parasitize internal

organs and tissues than the bovine strain. Whether the differences have a genetic basis or are a result of adaptation of the bovine strain to goats by an evolutionary process is unknown. There is no reference in the literature on the antigenic structure of *Besnoitia*. Consideration of this account makes it apparent that *Besnoitia* found in goats in Kenya is a specific strain or a biological variant of *Besnoitia*, different from *B. besnoiti*, the cause of bovine besnoitiosis. The name *Besnoitia granulomae* is proposed for the *Besnoitia* spp. isolated from goats in Kenya.

## CONCLUSIONS

Caprine besnoitiosis has been diagnosed in flocks of goats in Eastern, Rift valley and Coast provinces of Kenya.

Rabbits are refractory to the strain of *Besnoitia* afflicting goats in Kenya.

Natural, chronic cases of caprine besnoitiosis are unlikely to serve as reservoirs of bovine besnoitiosis as is known today.

There appears to be a clinicopathological difference between the expression of *Besnoitia* strains infective to cattle and goats in Kenya.

Caprine besnoitiosis in Kenya is caused by a strain of *Besnoitia* different from *B. besnoiti*, the cause of bovine besnoitiosis. The name *Besnoitia granulomae* is proposed for the caprine strain.

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Year	Author	Title	Journal	Volume	Page
1960	Woodworth, H.C. and Weinman, D.	Studies on the toxin of <i>Toxoplasma</i> (toxotoxin)	Journal of infectious diseases	107	318-324
1992	Yerusham, I.; Tromp, A. and Pipano, E.	A case of clinical besnoitiosis in a 15 month old Israeli friesian calve	Israel journal of verinary medicine	47(4)	164

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## APPENDICES

APPENDIX 1: Mean body temperature (°C) in goats and rabbits infected with bovine *Besnoitia* cyst; bovine *Besnoitia* proliferative organisms; and caprine *Besnoitia* cyst organisms.

Days	Type of inoculum				
	I	II	III	IV	V
Pre-infection -5	38.8	38.8	39.3	38.8	39.5
-4	38.8	38.8	39.4	38.8	39.4
-3	38.8	38.7	39.5	38.9	39.6
-2	38.9	39.0	39.3	38.8	39.5
-1	38.9	39.0	39.4	39.0	39.5
*Day 0	38.7	38.8	39.3	38.9	39.7
Post-infection 1	38.7	38.8	39.3	39.0	39.6
2	38.8	39.3	39.3	38.8	39.7
3	39.0	38.4	39.1	38.9	39.5
4	38.9	38.4	39.5	39.0	39.5
5	39.0	38.5	39.7	38.9	39.8
6	39.5	38.7	39.5	39.2	39.7
7	39.5	39.4	38.4	39.2	39.4
8	39.9	41.3	39.4	39.5	39.4
9	40.3	40.7	39.8	39.6	39.5
10	40.1	41.1	40.2	39.9	39.7
11	40.9	40.9	39.7	40.8	40.5
12	40.7	40.7	40.2	40.7	39.9
13	40.5	39.4	39.9	40.5	39.7
14	39.7	39.4	40.5	40.5	40.2
15	39.7	38.0	40.3	39.9	39.9
16	40.1	38.8	40.1	40.0	40.2
17	40.0	39.0	40.1	39.6	39.8
18	40.2	38.8	40.4	39.5	39.6
19	39.3	38.7	40.4	39.2	39.7
20	38.7	38.7	40.1	39.3	39.6
21	38.7	38.8	39.9	39	39.6
22	38.8	-	40.3	38.8	39.5
23	38.6	-	40.4	-	39.5
24	38.6	-	39.7	-	39.6
25	-	-	40.2	-	39.5
26	-	-	40.2	-	-
27	-	-	40.0	-	-
28	-	-	39.7	-	-
29	-	-	39.5	-	-
30	-	-	39.3	-	-

Key: Column I: Bovine cyst organisms to goats, n=4.  
 Column II: Bovine proliferative organisms to goats, n=2.  
 Column III: Bovine proliferative organisms to rabbits, n=3.  
 Column IV: Caprine cyst organisms to goats, n=3.  
 Column V: Caprine cyst organisms to rabbits, n=3.

\* Day of infection

APPENDIX 2: Mean WBC ( $10^9/l$ ) in goats and rabbits infected with bovine and caprine *Besnoitia* cyst or proliferative (blood) forms.

Days pre-and post-exposure						
Source of organism	Inoculum type	-5	0	7	14	21
Bovine to goats	Cysts	16.56	17.84	20.21	16.60	18.02
	Proliferative form	16.66	16.48	18.95	23.25	24.86
Bovine to rabbits	Proliferative form	9.78	9.60	9.24	8.54	9.46
Goat to goat	Cysts	14.56	14.97	20.22	21.90	21.40
Goat to rabbits	Cysts	13.77	13.25	13.92	13.98	14.28
Control rabbits		11.75	10.21	10.34	11.33	10.21
Control goats		13.27	14.16	13.41	15.33	14.34

APPENDIX 2 Continued:

Days post-exposure									
28	35	42	49	56	63	70	77	84	91
17.72	21.97	20.31	18.71	15.29 <sup>a</sup>	13.36 <sup>a</sup>	12.71 <sup>a</sup>	14.30 <sup>a</sup>	14.22 <sup>a</sup>	-
22.52	22.76	20.11	21.80 <sup>a</sup>	-	-	-	-	-	-
11.36	11.52	13.40	13.71	13.21	14.62	13.91	14.67 <sup>a</sup>	-	-
20.24	19.74	18.27	18.32	19.14	19.45	17.21	10.82	18.87	15.35
14.56	11.77	9.02	9.40	7.81	10.72	13.77	5.18	9.90	12.74
6.71	9.67	10.86	9.82	9.98	10.02	10.30	11.67	9.42	9.77
15.71	18.62	16.42	14.62	14.32	13.11	9.56	9.72	10.11	10.62

Key. a). <sup>a</sup> = values from single animal

b). Normal ranges and means ( $10^9/l$ ) (Schalm *et al.*, 1975):

Caprine 4.0 - 13.0 (9.0)

Rabbit 3.2 - 23.5 (7.0 - 9.0)

APPENDIX 3: Mean PCV (%) in goats and rabbits infected with bovine and prine *Besnoitia* cyst or proliferative forms.

		Days pre-and post-exposure				
Source of organism	Inoculum type	-5	0	7	14	21
Bovine to goats	Cysts	26	24	22	31	23
	Proliferative form	23	23	21	24	20
Bovine to rabbits	Proliferative form	41	41	38	35	39
Goat to goat	Cysts	25	24	25	24	24
Goat to rabbits	Cysts	40	41	41	40	41
Control rabbits		40	42	40	41	41
Control goats		29	28	28	26	28

APPENDIX 3: Continued.

Days post-infection										
	28	35	42	49	56	63	70	77	84	91
21	20	19	20	20*	20*	20*	15*	19*	-	-
21	21	21	22*	-	-	-	-	-	-	-
38	35	44	38	38	36	40	39*	-	-	-
25	24	24	20	22	25	26	22	25	26	26
35	42	42	35	36	40	36	39	45	46	46
40	40	42	43	40	42	38	40	42	40	40
25	25	26	25	25	28	26	26	25	24	24

Key. a). \* values from single animal

b). Normal ranges and means (%) (Schalm *et al.*, 1975):

Caprine 22 - 38 (28)

Rabbit 40.3 - 43.3 (41)

## COMPARATIVE STUDY ON CAPRINE AND BOVINE BESNOITIOSIS: INFECTIONS IN GOATS AND RABBITS

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### ABSTRACT

Investigations were carried out to study the disease symptoms that developed from two *Besnoitia* strains isolated from chronic natural cases of bovine and caprine besnoitiosis, ~~were experimentally investigated~~. Each isolate was used to infect a set of experimental hosts comprising both weaned local goats and Newzealand white rabbits.

Two distinct clinical syndromes were observed which revealed obvious differences in pathogenicity between the two isolates. Recipients of the bovine isolate developed typical signs of acute besnoitiosis often leading to deaths before the chronic disease could establish. Deaths in rabbits occurred within 18 hours after infection with bovine *Besnoitia bradyzoites*. Recipients of the caprine isolate developed a mild disease only marked by development of fever after incubation period of 10-14 days and chronic cysts in the caprine hosts. It is proposed that *Besnoitia* organisms causing besnoitiosis in goats and cattle should be considered as separate strains of *Besnoitia besnoiti*.

### INTRODUCTION

Besnoitiosis is an acute, subacute or chronic disease caused by coccidia protozoan parasites of the genus *Besnoitia*. *Besnoitia* parasites are classified together with *Isospora coccidia* of the genera *Toxoplasma*, *Hammondia* and *Cystoisospora* in the subfamily Toxoplasmatinae, family Sarcocystidae (Frenkel, 1977). The disease is characterised by the formation of chronic miliary cysts in all parasitised organs or tissues that contain blood vessels (Bwangamoi, 1979). The acute clinical syndrome, which follows an incubation period of upto 13 days, is characterised by pyrexia, anorexia, general malaise, enlargement of superficial lymph nodes, lacrimation, anasarca, alopecia, rhinitis, pharyngolaryngitis, tracheitis and orchitis. The clinical syndrome is less marked in subacute infections and the chronic form is more commonly diagnosed in nature. The cysts are commonly encountered in the skin, subcutis, skeletal muscles, intermuscular fascia, mucosae of the anterior respiratory tracts, synovial sheath, periosteum and testis. Degenerative and necrotic changes, particularly in the skin and testis precede development of cysts although usually no internal gross pathological lesions directly referable to besnoitiosis are encountered at this stage. Cyst development in antelope infections commonly occurs more in the internal organs (Cheema and Toofanian, 1979; McCully et al. 1966). Tentative diagnosis is based on the seasonal occurrence of besnoitiosis, clinical symptoms and lesions at necropsy (Pols, 1960) but examination of cysts in the sclero-conjunctiva and the anterior nares provides a fast diagnostic method (Bigalke and Naude, 1962). Histological examination readily reveals typical cysts in infected sections. *Besnoitia* antibodies are demonstrable by various serological methods most of which are hardly specific.

It is not clear whether *Besnoitia* organisms found in various animal species belong to one species with different strains or are different species. The speciation of the genus is still based on: (a) the species of experimental animals infected, (b) the degree of infection produced in hamsters, (c) the differences in cyst size, (d) the pathology found in the natural host, and (e) factors of geographical incidence (Suggs et al. 1968). Differences in virulence in various hosts (Bigalke et al. 1967; Basson et al. 1970) and isoenzyme electrophoresis (Le Blancq et al. 1986) have been applied to differentiate

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bovine and antelope strains of ~~*Besnoitia besnoiti*~~ *Besnoitia besnoiti* (Marotel, 1912) is associated with disease in bovine hosts and is thought to cause natural caprine besnoitiosis leading to similar clinical manifestations and pathology. Rabbits are very susceptible to infection with proliferative (Pols, 1954, 1960) and cyst forms (Bigalke 1960, 1967) of *Besnoitia besnoiti* of cattle and serve as good experimental models for studies on bovine *Besnoitia besnoiti* infection.

Although spontaneous besnoitiosis in domestic goats has been reported in Kenya (Bwangamoi, 1967; Bwangamoi, et al. 1989) and Iran (Cheema and Toofanian, 1979) little is known about caprine besnoitiosis and the identity of *Besnoitia* parasites found naturally in goats is yet to be clarified. Recent observations negate previous assumption that one strain of *Besnoitia* was responsible for besnoitiosis in both cattle and goats. In one natural outbreak of caprine besnoitiosis (Bwangamoi, et al. 1989), no trace of besnoitiosis was found in cattle and sheep which had grazed alongside the goats for period of two years. Such an involvement was, however, not completely ruled out since no serological tests were done on the cattle. Nevertheless, even if such tests found cattle positive, it would still raise the question why the disease suddenly manifested in hundreds of goats amongst other animals. A flock of goats admitted to the Department of Veterinary Pathology and Microbiology, University of Nairobi, expressed a typical besnoitiosis enhancing speculation on the existence of a species of *Besnoitia* in goats different from *Besnoitia besnoiti* (Marotel, 1912), the cause of bovine besnoitiosis. This paper comprises findings obtained in studies on the relationship between clinical disease syndromes following artificial infections with bovine and caprine isolates of *Besnoitia besnoiti*.

## MATERIALS AND METHODS

Procurement, preparation and demonstration of *Besnoitia* parasites. The initial bovine *Besnoitia* parasites were isolated from skin cysts of cattle with chronic besnoitiosis at the Agricultural Development Corporation (ADC) Galana Ranch, Coast Province. Caprine *Besnoitia* parasites were isolated from superficial and intermuscular fascia of a goat with chronic besnoitiosis brought from the Coast province. The bovine skin scrappings and sections of infected fascia from the goat were collected and transported into bottles containing phosphate buffered saline (PBS) pH 7.2 containing 200 units/ml benzylpenicillin sodium (Dawa Pharmaceuticals, Ltd. Nairobi) 200ug/ml streptomycin sulphate and 50ug/ml Nystatin (Squibb). The material was crushed in a mortar and pestle (Bigalke, 1967) to free parasites from the cysts and filtered through whatman filter paper number 91 (Whatman Ltd, Maidstone, England) to separate parasites from the larger cell debris. The filtrate was washed three times in PBS by centrifugation at 2000 rpm for 10 minutes, the supernatant discarded and the resulting pellet resuspended to an appropriate volume in PBS before it was treated to form the inoculum. An estimate of live parasite concentration in the inocula was determined by counting on an improved Neubauer haemocytometer (Assistant, W. Germany) while simultaneously checking for motility. In all cases Giemsa smears were prepared and examined for *Besnoitia* parasites at x400 magnification.

### Experimental Animals

Five months old New Zealand white rabbits, weighing about 2kg were used. They were obtained from coccidia-free stock bred at Kabete. Either sex was used. They were housed singly in wire cages at room temperature with clean water, rabbit pellet and vegetable supplement provided *ad libitum*. Local goats, between 6 and 8 months old, were obtained from besnoitia-free area and therefore assumed not to be infected with *Besnoitia besnoiti*. Physical examination was done to determine absence of the obvious cysts in the scleroconjunctiva. They were housed in a concrete floor stockade under tick and fly-free conditions at the Veterinary Laboratories, Kabete. They were provided with clean water and red oat hay *ad libitum*, commercial feed (Unga cubes, Unga Group

(K) Ltd.), and Maclick mineral supplement (Cooper (K) Ltd., Nairobi) were also provided. The goats were dewormed and also washed with acaricide two weeks before infection.

### *Experimental Design and Disease Study Procedures*

The rabbits were infected with the *Besnoitia* parasites as follows: Cyst organisms of bovine *Besnoitia besnoiti* were used to infect 8 rabbits, R103, R101, R104, R148, R111, R109, R123, R150. Proliferative organisms of goat-passaged bovine *Besnoitia besnoiti* were used to infect 3 rabbits R154, R156, R161. Cyst organisms of <sup>caprine</sup> *Besnoitia besnoiti* were used to infect four goats He1, He2, He3, He4. Two goats He5 and He6 received transfusions from goats infected with recently isolated bovine strain during the acute phase. Cyst organisms of caprine *Besnoitia besnoiti* were used in the infection of 3 goats Ho1, Ho2, Ho3. \* Another group, consisting of two rabbits, R126 and R128 and two goats, Gc1, Gc2 were kept as controls after receiving similar pre-infection treatment. Rabbit R126 and goat Gc1 each received a sterile injection of 20 ml on PBS simultaneously. Infected animals were constantly examined for signs of besnoitiosis. Blood, lymph node smears and rectal temperatures were taken daily during the period immediately before and after infection and through the acute stage of the disease. \* and three rabbits R106, 102 and R109.

## RESULTS

### *Infections with Bovine Cyst Organisms*

Observations were made on four goats and eight rabbits infected with bradyzoite forms of bovine *Besnoitia besnoiti*. Acute reaction were obtained in the goats after an incubation period that varied from 8 to 13 days when temperature rise was detected. Pyrexia persisted for 5 to 11 days and fluctuated between 39.8 and 41.1C (Fig. 1). Enlargement of superficial lymph nodes occurred first around the infection sites, 2 to 3 days before the temperature rise and later affected others at a variable degree. Some became obviously very oedematous as the disease progressed. Dejection, trembling, and anorexia gave an early ill appearance. Lacrimation, rhinitis oedema, swelling on the face, oedema of the cornea, eyelids, ears, lips, palate, the tail and the vulva constituted accompanying signs at, or shortly after the peak of the febrile reaction. What followed was an extension of these signs especially oedema which was the most distinct symptom. The ocular and nasal discharges changed from serous to mucopurulent due to secondary conjunctivitis, rhinitis and pneumonia. The latter precipitated a situation of respiratory distress, sometimes severe with occasional moist coughs. Engorgement of the scleral blood vessels was a constant symptom and occasional petechiae and haemorrhages appeared on the palate and the vulva. Oedema extended to the neck, brisket and the legs and became very pronounced leading to a general state of anasarca, a rough hair coat and recumbency. In two cases the heads became very enlarged and heavy causing a lot of distress. These two goats, He1 and He2 were permanently recumbent, completely anorexic and developed sub normal temperatures before they died on day 21 and 20 post infection, respectively. Two other goats He3 and He4 had the primary acute reactions subsiding 4 to 5 days after temperature returned to normal although He4 was treated with sulphadimidine to prevent imminent death at this stage. This phase however, was marked by weakness and general emaciation as oedema subsided. The hyperaemia on the sclera also waned but the conjunctiva developed rough appearance, an early sign of cyst development. In one case, (He4) alopecia developed over the neck, flanks, the medial aspects of the limbs and the posterior parts of the body. Developing cysts visibly appeared on the conjunctiva of the goat He3, 30 days after termination of pyrexia but the goat died of besnoitiosis 5 days after extreme emaciation. He4 was sacrificed at the end of the study period. The extensive skin reaction so characteristic of chronic besnoitiosis in cattle did not develop although none of the goats survived long enough to exhibit a truly chronic phase. During the acute phase, extracellular parasites were only occasionally demonstrated on smears of oedema fluid, blood and lymph aspirates but besnoitiosis



was confirmed by sub inoculation of blood collected at the peak of pyrexia to two goats and two rabbits. Anasarca was the major internal lesion in the two animals that died during the acute phase. The lymph nodes, lungs, liver, and the spleen were enlarged with oedema fluid. There was a general increase of fluid in the body cavities. Subcutaneous and superficial blood vessels were remarkably engorged, especially around the head, neck and the limbs where oedema was more extensive. Carcasses of two goats that died after the acute phase were extremely emaciated but had no other pathological changes referable to besnoitiosis. Eight rabbits infected with cyst bradyzoites by various parenteral routes, intravenous, intraperitoneal and subcutaneous died overnight following infection. No significant lesions were detected at postmortem other than hyperaemia at the site of injection. This included one rabbit given 3 days pre-infection treatment with 2% sulphadimidine in drinking water.

#### *Infections with Bovine Proliferative Forms (Trophozoites)*

An acute syndrome, similar to that described of goats infected with cyst organisms was expressed in two goats infected with proliferative organisms. The incubation period was reduced to 7 days and pyrexia lasted for 6 and 8 days getting to a peak of 41.4°C (figure 1). At the height of the fever the animals were visibly ill, listless and dejected. Both reacted severely with oedema over the face, neck, legs and conjunctiva, lacrimation, mucoid nasal discharge and dyspnoea. Trembling, enlargement of superficial lymph nodes, occasional moist coughs and hyperaemia of the sclera accompanied the febrile reaction. Oedema extended to the testicles causing a remarkable enlargement and pain. At the termination of the acute phase, extreme emaciation preceded death 52 and 48 days post infection. Extracellular parasites were demonstrated only occasionally on lymph aspirate, plasma and particularly in the testicular oedema fluid during the pyrexia phase. The presence of *Besnoitia besnoiti* was better proven when blood collected at the peak of the fever was sub-inoculated into a rabbit which developed a severe reaction.

Rabbits developed equally prominent clinical symptoms during the febrile stage. A well defined thermal reaction was expressed in all three rabbits infected with proliferative forms in blood. Fever persisted for 15 days and longer in one instance and ranged between 39.6°C to 41.2°C (Figure 1). During the fever, the animals were listless, anorexic, and lost weight rapidly. At about the peak of reaction, hot painful, subcutaneous oedematous swellings especially of the ears, eyes, head and limbs developed. The males developed testicular and scrotal oedematous swellings and severe congestion which progressed to complete necrosis of scrotum and testis. In one case, the scrotum opened into an ugly wound causing a lot of distress. One rabbit R154 developed severe oedema, congestion, skin necrosis and gangrene on the ears which sloughed off to stumps. Serosanguinous exudate formed into crusts and scabs that bled easily on the ears. Congestion and gangrene occurred on the lower parts of the limbs, the phalanges, and over the nose. Hyperaemia of the sclera and lacrimation developed in all the animals within this period. Serous to mucoid nasal discharges caused a marked respiratory distress. A severe skin reaction in two cases R156 and R154 showed signs of developing into advanced scleroderma often seen in cattle when depilation occurred over areas that were previously swollen with oedema. One rabbit succumbed to the distress and extreme emaciation and died on the 42nd day. Another was sacrificed on the 73rd day to alleviate further suffering while the third survived the study period. A leucocytosis was a characteristic symptom inferred readily from ordinary blood smears during the inflammatory reaction. Anaemia became apparent in blood smears at about the peak of reaction exhibiting hypochromatic macrocytes. Extracellular parasites were occasionally seen on stained blood smears and wet mounts of oedema fluid and the serosanguinous skin exudates from the ears. Apart from one rabbit, R161, all animals in this group died during the acute phase reactions. Anasarca and cachexia were again the major gross pathological lesions. One goat showed petechial haemorrhages and grayish thrombi under the skin of the limbs. Haemorrhages were encountered on the lungs in one rabbit.

### *Infections with Caprine Cyst Organisms*

Clinical symptoms exhibited by the 3 goats infected with caprine cyst bradyzoites did not present any characteristic features. For a few days pyrexia, anorexia and superficial lymphadenopathy were the only observable symptoms. Fever persisted for an average of 8 days, rising to a peak of 41.2°C and subsiding gradually to normal (Figure 2). Enlarged lymph nodes regressed soon after the decline of pyrexia. One animal developed a slight nasal discharge for two days. Two to 3 weeks after the febrile phase, cysts development was detected on the sclera conjunctiva causing an uneven appearance in the eyes and slight lacrimation. Four days later, the cysts were quite prominent and solid. One animal developed slight oedema on the lips vulva, and eyelids but this quickly disappeared. However, this animal died 8 days later to unrelated cause. A large ulcer and perforation of the rumen found at postmortem were not related to besnoitiosis. The two animals developed typical chronic besnoitiosis and survived the study period without any deterioration in body condition. The reaction in 3 rabbits in this group were mild. Only pyrexia was seen after an incubation period of about 11 days. The febrile reaction lasted for an average of 5 days when temperature ranged between 39.8°C and 41.5°C subsiding gradually to normal (Figure 2). No further outward disease manifestations were observed on this group, all of which survived the study period in very good body condition.

No parasites could be demonstrated in the blood smears of any of the animals in this group during the febrile phase nor could cysts be seen in the 3 rabbits. However, chronic besnoitiosis in the goats was demonstrated by the extensive development of typical *Besnoitia* cysts in the eyes. At autopsy, no internal pathological lesions directly referable to besnoitiosis were observed, with the exception of the presence of cysts in the goats visible as opaque, whitish raised nodules in the subcutis, superficial fascia and aponeurosis of the muscles without evidence of any inflammatory reaction. Cysts were mainly encountered around the neck, limbs, the gluteal muscles, the back along the spine, the visceral aspect of the thoracic wall in one case and rarely on the abdominal surface. In the same areas, white streaks were observed in the muscles which could be cysts. Macroscopically, no nodules as seen in goats could be detected in the subcutis, fascia, sclera, and nasal, pharyngeal and tracheal mucous membranes in the rabbits.

### DISCUSSION AND CONCLUSIONS

The main objective of this investigation was to determine whether *Besnoitia* found in goats was related to *B. besnoiti* of cattle. Parasites of the genus *Besnoitia* are very similar morphologically and cyst organisms are indistinguishable. These were no exception. Contrary to expectations, two sets of results were obtained from the two parasites. *Besnoitia* of goats behaved different in rabbits and goats from that observed with bovine isolates. Typical skin and testicular lesions were absent. Proliferative organisms were also absent in blood smears of goats and rabbits that received the caprine isolate but chronic cysts were detected on the sclera of the goats 2-3 weeks after the febrile phase. The absence of typical lesions of besnoitiosis in rabbits that received the caprine strain has not been reported previously. The bovine strain of *B. besnoiti* was more pathogenic to rabbits and goats than the caprine strain. Rabbits and goats developed typical symptoms of besnoitiosis as described by Basson et al. (1970) and Pols (1960). Skin lesions in the rabbits were severe. Lesions in the male genital organs were characteristic and included oedema and necrosis. The febrile reactions were more pronounced and lasted longer when goats and rabbits were infected with bovine strains organisms, although proliferative forms could be found in blood slides only occasionally. Sub-inoculation into rabbits and other goats proved that they had been present in the blood during the reaction. Only one of artificial transmission of besnoitiosis from chronically infected cattle to goats has been recorded before (Pols, 1960). One goat out of four infected reacted severely.



It is notable that 8 rabbits injected with bovine strain cyst organisms via different parenteral routes died within 24 hours. Evidence of the toxicity of cyst forms to rabbits has been provided before (Bigalke, 1967). This is irrespective of the route of administration. In these investigations, no such observation was made when rabbits were injected with the same number of cyst organisms of the caprine strain. Further and more precise investigations on this 'besnotoxin' will be necessary before conclusions can be made.

It is significant that the caprine strains of *B. besnoiti* were only very mildly pathogenic to rabbits and produced subclinical infections in goats only marked by an extensive development of chronic cysts. A striking difference between the rabbits which were injected with bovine and caprine strains was the absence of testicular and skin lesions in the latter as compared to their severity in the former. Goats that received either cyst or proliferative organisms of the bovine strain rarely survived the acute phase although observable cysts developed in the eyes of one case. The disease terminated fatally in all but one goat and one rabbit that received the bovine strain in either form. Although factors governing the pathogenicity of *Besnoitia* are yet to be understood, the findings clearly illustrate biological differences between the caprine and bovine strains. Whether the differences have a genetic basis or are a result of adaptation of the bovine strains to goats by an evolutionary process is unknown. It does, however, appear that a specific strain or biological variant of *Besnoitia* causes natural infection in goats and should be considered different from *B. besnoiti*, the cause of bovine besnoitiosis.

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**Fig. 1 RESPONSE OF GOATS AND RABBITS TO BOVINE  
BESNOITIA CYST AND PROLIFERATIVE FORMS**

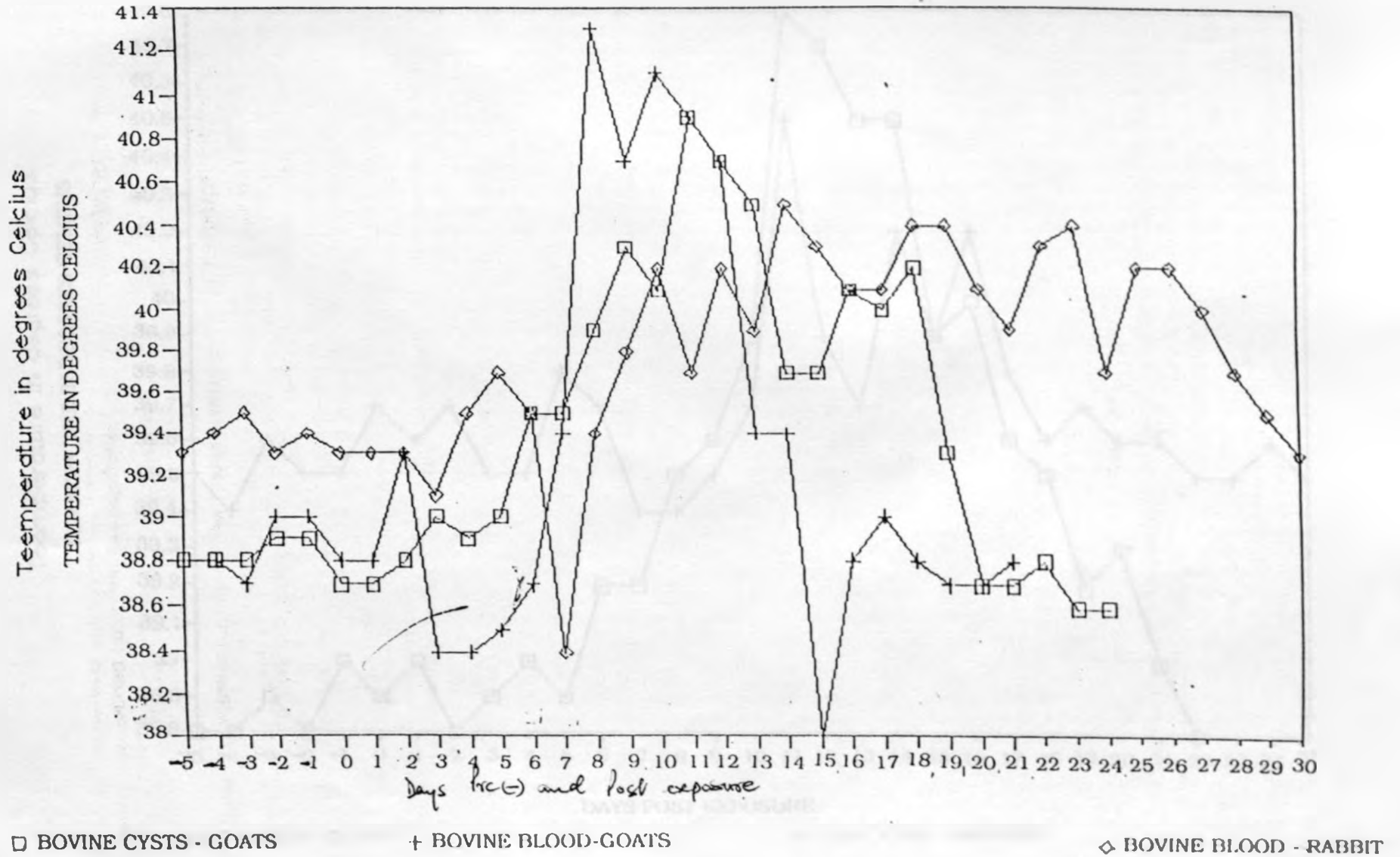


Fig. 2 RESPONSE OF GOATS AND RABBITS TO GOAT

