A COMPARATIVE STUDY ON NATURAL AND EXPERIMENTAL CAPRINE AND BOVINE BESNOITIOSIS

BY

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University

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DEDICATION

This thesis is dedicated to my late grandparents

NJENGA MUCHUKU and GATHUIYA NJENGA

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ABSTRACT

A survey on the incidence of besnoitiosis in the domestic ruminants in Kenya was conducted for two years. A total of 29 districts were visited and 38,577 goats, 16,014 cattle and 14,818 sheep were examined for cysts in the eye as recommended by Bigalke and Neude, (1962). To confirm diagnosis skin biopsies were taken and histopathology done.

The survey showed that besnoitiosis was most important in goats, followed by cattle, while sheep were free. Caprine besnoitiosis occurred in a continuous belt from the Coast, North Eastern, Eastern, Southern Rift Valley, Nairobi and Northern Rift Valley. The highest infection rate was found in Mandela - 36.8 per cent; followed by Kwale - 35.2 percent; Isiolo - 34.8 percent; Marsabit - 33 percent; Wajir - 28 percent; Nairobi 25.6 percent; Meru -24.4 percent; Garissa - 21 percent; Taita Taveta - 18 percent, Embu - 16.7 percent; Kitui - 8.6 percent; Machakos - 6.5 percent; Laikipia - 2.8 percent, Kajiado - 1.6 percent; Turkana - 0.9 percent and Elgeyo-Marakwet - 0.8 percent. In all districts (except Nairobi) where caprine infection rates were above 6.0 percent kids were infected.

Bovine besnoitiosis was only found in Tana River district where an infection rate of 11.1 percent was found. Goats in this district were free from besnoitiosis. Sheep from all the provinces were found to

be free from besnoitiosis.

Acute besnoitiosis, hitherto unreported was observed in goats. They had fever (>40.5°C), anorexia, lacrimation, dull demeanour and anasarca. In bucks the testis were swollen and painful to touch. Chronic caprine besnoitiosis was characterised by alopecia, hyperkeratosis and scleroconjuctival cysts. The following semen characteristic indices were found to be low in the chronically sick bucks; Sperm counts (0-$1.5x10^6$); Individual sperm motility (0-80 percent) and live spermatozoas (0-85 percent). The semen also had no motion of swirl and the sperms had a high defect rate (50 percent).

Gross and histopathology revealed that large number of cysts occurred in the subcutis, intermuscular fascia of superficial muscles, ligaments, tendons and periosteum of the lower limbs, upper respiratory tract including trachea, pampinform plexuses and peripheral blood vessels.

Study on the staining characteristics revealed that Haematoxylin and Eosin (H & E) was the best general stain for demonstrating cysts, while Giemsa, Van Gieson (VG), Periodic Acid Schiff (PAS), Toluidine Blue and Fulgen were good for demonstrating the cyst wall; Giemsa and PAS were good for demonstrating host cell; PAS, Toluidine Blue and Fulgen for demonstrating cystozoites while DNA/RNA Pyronin for demonstrating areas of the cyst showing cystozoites which had wall forming body 1 (W1) on examining the cysts with the electron microscope.

Cattle, goats, rabbits, mice, rats, guinea pigs and hamsters were inoculated with cystozoites from a chronically sick goat. All the animals except the goats were refractory to caprine besnoitia. Other cattle, goats and rabbits were inoculated with cystozoites of B. besnoiti from a chronically sick cow. All of them got infected, with the rabbits showing a more severe form than cattle. Thus the caprine besnoitia was found to be specific to the goat while B. besnoitia affects cattle as well as other animals.

The availability of cattle, rabbits and goats that were previously exposed to caprine besnoitia and the availability of B. besnoitia organisms made it imperative to check whether these animals could be experimentally infected with B. besnoiti. Inoculation of large number of cystozoites of B. besnoiti into these animals did not result in infection.

Inoculation of goats (2 for every route) using cystozoites from chronically infected goats through the intravenous, subcutanous, intraconjuctival and the nasal routes resulted in infection in the recipient animals. Infection was also achieved through alternate needle pricking from chronically sick to healthy goats and through subcutanous implantation of intact fascia from a chronically sick goat into healthy ones. Attempts to reproduce the disease through the intrauterine, oral and ocular routes was not successful. Thus the two Besnoitia species behave similarily in terms of infection routes.

Serial studies on the development of the disease in goats showed that after inoculation of cystozoites into the receipient animals endothelial and histiocytic cells were invaded. The invaded cells became enlarged and multinucleated. The merozoites multiplied in the host cells by endodyogeny until they burst the cells. The released merozoites invaded other cells further away and the cycle was repeated. Cyst formation occurred concurrently with this cycle of replication. Mature cysts were also found to release cystozoites in chronic cases leading to young cysts being formed. This has not been reported in bovine besnoitiosis. The presence of circulating merozoites (bradyzoites) was associated with the acute form of the disease.

The maturity of the cysts took approximately 112 days. Young cysts had thick cyst walls with viable host cell nucleus. Mature cysts had thin cyst walls with flattened host cell nucleus.

Comparative ultrastructural studies revealed that the host cell of B. besnoiti had a high lipid content and a lot of rough endoplasmic reticulum. The cystozoites had a wavy pellicle and the outer cylindrical structure of the micropore was formed purely from the inner membrane of the pellicle. The wall forming body one (Wl) was found to be a large membrane bound body with small membrane bound bodies in it . The microtubules after originating in the apical ring of the cystozoite were found to move straight for the first one sixth of the organism then spiral and

terminate at the posterior ring. It was also found that B. besnoiti cystozoites had in most cases nucleii with intact nuclear membrane and granular chromatin. Other easily demonstrable organelles (ribosome like structures and mitochondria) were also found in the cytoplasm of the cystozoites.

Caprine besnoitia on the other hand had a host cell with low lipid, high polysaccharide content, many tubular mitochondria and little rough endoplasmic reticulum. The cystozoites of caprine besnoitia had a smooth pellicle and the outer cylindrical structure of the micropore was formed from the inner membrane of the pellicle and an outgrowth from the outer membrane of the pellicle. The wall forming body one (W1) was a dark dense homogenous body with a limiting membrane, while the microtubules after originating from the apical ring were straight until they terminated in the posterior ring. All the cystozoites of caprine besnoitia had nucleus with no nuclear membrane and a relatively smooth (homogenous) chromatin. Large amounts of amylopectin (polysaccharide) were found in the cytoplasm of the cystozoites and it was also difficult to demonstrate other organelles (ribosomes and mitochondria).

It was thus clear from field surveys, natural and experimental studies, pathogenesis and ultrastructure that caprine besnoitia is different from B. besnoiti. The name Besnoitia caprae is suggested for the goat pathogen.

CHAPTER 1

1.0 INTRODUCTION

Besnoitiosis is a disease of domestic and wild ruminants, equines, rodents, opossums and lizards caused by various species of the cyst forming protozoan parasites of the genus Besnoitia. Among the known species are Besnoitia besnoiti of cattle and wildebeest, B. wallacei of rats and mice, B. jellisoni of rats, mice and hamsters, B. darlingi of lizards and opossums, B. benetti of horses and donkeys and B. tarandi of the caribou and reindeers.

Bovine besnoitiosis was first reported in Southern France in 1912 by Besnoit and Robin (Marotel, 1912). Since then it has been reported in Portugal, South Africa, Swaziland, Botswana, Namibia, Zimbabwe, Angola, Kenya, Tanzania, Uganda, Zaire, Sudan, Cameroon, Nigeria, Israel, former U.S.S.R., South Korea and Venezuela. Among these countries the level of endemicity is only known in South Africa, Namibia, Zimbabwe, Israel and former U.S.S.R. but not the others (Bigalke, 1981).

Since the disease was first reported, many studies have been conducted on its aetiology (Pols, 1960), transmission (Pols, 1954a,b, Bigalke, 1967, 1968, Peteshev, Galuzo I.G. and Poloshnov, A.P., 1974), pathogenesis (Basson, McCully and Bigalke, 1970), chemotherapy (Shkap, V., Marcowitz, A., Pipano, E., Greenblath, C., Dewaal, D.J., Potgieter, F.T. and Unga-Waron, H. 1982, 1985,1987), diagnosis (Bigalke and

Neuae, 1962), immunology (Bigalke, Basson, McCully, Bosman and Schoeman, 1974, Kaggwa, E., Weiland, G. and Rommel, M. 1979, Shkap, Unga-Waron, Pipano and Greenblath, 1984), pathology (Schulz, 1960, McCully, Basson, Van Niekerk and Bigalke, 1966, Basson, et. al., 1970, Neuman, M., Nobel, T.A. and Perelman, B.Zo 1979, Neuman and Nobel, 1981, Kumi-Diaka, Wilson, Sannusi, Njoku, and Osori, 1981, Nobel, Klopfer, Perl, Nyska, Neuman and Brenner, 1981) and ultrastructure (Neuman, 1974, Gobel, E., Widaur, R., Rumann, M. and Munz, E. 1985 and Shkap, Yakobson and Pipano, 1988).

The cystozoites of Besnoitia besnoiti are crescent shaped and measure on average 8.4 x 1.9µm (Bigalke, 1981). Their ultrastructural features at the cyst stage are typical of apicomplexa with a polar ring, conoid microtubules, rhoptries and micronemes (Neuman, 1974).

Naturally B. besnoiti infects cattle and wildebeest but experimentally can infect rabbits (Pols, 1960, Bigalke, 1968), white mice and gerbils (Neuman, 1962b), sheep and goats (Pols, 1960) and hamsters (Bigalke, 1968).

Based on observations made on other Besnoitia spp. (reviewed by Dubey, 1977) and a single yet unconfirmed investigation on B. besnoiti (Peteshev et al., 1974), it was thought that cattle contract the disease by ingestion of mature isosporan-type oocysts shed in feaces of members of the cat family (Bigalke, 1981).

The sporozoites which emerge from the oocysts enter circulation and multiply by endodyogeny in endothelial

cells of blood vessels, particularly in the dermis, subcutis, fascia and upper respiratory tract (Basson, et. al., 1970). Merozoite-like endozoites (=tachyzoites) emerge from the damaged cells to re-invade adjacent or more distant cells to produce further endozoites. This cycle is superceded by cyst formation approximately one week after the initial cycle of replication (Basson, et. al., 1970). The activated histiocytic cells containing the merozoite like cystozoites (=bradyzoites) in or in close association with blood vessel walls become detectable in the same sites where the endozoites were formed. The cystozoites multiply by endodyogeny in the vacuoles of the markedly enlarging host cells with hyperplastic and hypertrophic nuclei. The characteristic thick walled cysts reach up to 400µm (Basson, et. al., 1970). Cyst formation is remarkably synchronous and there is no evidence that cystozoites from disintegrating cysts give rise to further cysts or endozoites in the same animal (Pols, 1960, Bigalke, 1968).

The disease in cattle has two distinct sequential stages namely the anarsarca stage and the scleroderma stage. The anarsarca stage is associated with proliferation of endozoites and is characterised by fever which appears 4 days after oral infection with sporulated oocysts of feline origin (Peteshev et al., 1974) or 13 days on parenteral (subcutenous) mechanical infection with cystozoites (Bigalke, 1968). The fever is usually between 40-41°C or higher and lasts for up to 7 days.

The fever is accompanied by progressive inappetence

followed by complete anorexia, weight loss, increased respiratory rate, photophobia, listlessness and the animals prefer recumbent positions and walk slowly when forced to. Hyperemia of the muzzle, periorbital and scrotal skin is noticeable in light coloured animals a few days after temperature rise. Anasarca which appears as filling of the face or thickening of skin folds of the neck, back and chest in mild cases or total thickening of the whole skin in severe cases appear about this time. The testes are swollen and painful on palpation.

The early anasarca may go unnoticed and its presence only revealed when fluid accumulates on the ventral parts of the body namely the intermandibular, brisket, sternal, abdominal, preputial, tail and limb regions. Occassionally the anasarca stage is not detected at all. There may be a distinct break between the anasarca and the scleroderma stage with disappearance of oedema by the end of the 3rd week, reduced temperature and appetite improvement or the merging of the two stages (Bigalke, 1981).

The scleroderma stage is characterised by progressive thickening, hardening, decreasing elasticity and consequential prominent folding and puckering of the skin. This appears 3 to 4 weeks after the rise in temperature. It is accompanied by progressive alopecia. The epidermis may be shed in patches leaving grey seborrheic areas, and sitfasts are commonly seen over bony prominences as well as on the scrotum of bulls. Deep raw maggot-infested fissures sometimes develop

between the skin folds especially of the breech and flexor surfaces of the joints. Seborrhoea is a characteristic chronic feature in all cases. Nodule formation with alopecia around the eyes and mouth occur in mild cases. The superficial lymph nodes are always swollen.

A mucopurulent nasal discharge, which forms crusts and clogs the nostrils is common during the scleroderma stage while a low level undulant fever may persist during the first few weeks of this stage. The animal is listless, has poor appetite and is emaciated. One or both testes become atrophic and indurated. Bulls develop permanent aspermatogenosis due to severe testicular lesions which develop 2 weeks after the initial clinical reaction (Pols, 1960). Conjuctival cysts become visible 6-7 weeks after the rise of temperature (Bigalke, 1968).

Death can occur in either of the stages, but most animals survive. However convalescence is slow and scleroderma and alopecia may become life long features. Clinically inapparent infections that go unnoticed by farmers represent the majority of cases in the field (Bigalke, 1968).

Although bovine besnoitiosis has low mortality the affected animals suffer severe loss of productivity (Bigalke, 1981). Hides from the affected animals are useless for tanning into leather (Bwangamoi, 1968).

In contrast to the many reports on bovine besnoitiosis there are a few reports on caprine besnoitiosis. These have only been from Kenya and Iran.

Bwangamoi (1967) reported the presence of B. besnoiti in goat skins from Baringo in Kenya, while Kaliner (1973) reported B. besnoiti in the lungs of a goat that had died of pneumonia. Heydorn, Senaud, Mehlhorn and Heinonen, (1984) described the ultrastructure of a goat besnoitia from Kenya which resembled B. besnoiti. Lately Bwangamoi (1989) and Bwangamoi et.al. (1989) reported an epidemic of goat besnoitiosis in the Eastern Province of Kenya. Cheema and Toofanian (1979) reported besnoitiosis in wild and domestic goats in the Far East province of Iran. All these reports were on natural chronic cases.

According to Bwangamoi (1967) and Cheema and Toofanian (1979) the cause of caprine besnoitiosis was B. besnoiti. However according to Bigalke (1981) the besnoitia that causes besnoitiosis in goats in Kenya and Iran is unidentified and its relationship with B. besnoiti is unknown. He also observed that its level of endemicity in both countries was unknown.

During the epidemic reported by Bwangamoi et. al. (1989), cattle reared together with infected goats for a period of 2 years did not have besnoitiosis. However three Dorper sheep were infected, thus seemingly substantiating the only other report of suspect besnoitiosis in sheep by Hicks (1982).

Clinically, skin ulcerations, dullness and anorexia were reported in a wild goat suffering from besnoitiosis in Iran (Cheema and Toofanian, 1979). Alopecia, presence of scleroconjuctival cysts, emaciation, staring-steely hair coat, dermatitis and necrosis of the scrotum with

terminal pneumonia was reported for goats in Kenya (Bwangamoi, et. al., 1989; Bwangamoi, 1989). Acute caprine besnoitiosis was yet to be reported.

Grossly, alopecia, hyperkeratosis, seborrhoea sicca and presence of large number of besnoitia cysts in the subcutis was reported (Cheema and Toofanian, 1979; Bwangamoi et al, 1989; Bwangamoi, 1989).

Microscopically in a wild goat presence of besnoitia cysts measuring 64 x 93 µm in the dermis and subcutis, 132 x 183µM in the epididymis, testis and blood vessels and 113 x 150µm in the lung and abomasum were observed. In another domestic goat the cysts ranged in size from 115 x 156µm to 217 x 414µm with an average of 175 x 290µm (Cheema and Toofanian, 1979). In Kenya the besnoitia cysts in the skin measured 150 x 310µm and 56 to 179µm in the testicles (Bwangamoi, 1989). In both reports complete aspermia was noted on histopathology in the bucks.

The staining characteristics of besnoitia cyst using haematoxylin and eosin (H & E), Masson's trichrome (MT), Periodic Acid Schiff (PAS) and Van Giesons's stains has been reported (Cheema and Toofanian, 1979).

Since no controlled studies have been conducted on caprine besnoitiosis little is known about its endemicity, aetiology and pathogenesis.

However according to Bwangamoi (1968) lack of previous reports, shortage of qualified personnel and flattening of cysts during drying of the skins may have been the reason for missing the diagnosis of this disease

in goats at an earlier date.

During a survey on diseases and defects that downgrade hides and skins in East Africa both bovine and caprine besnoitiosis were found to occur in Kenya. The goats that were affected came mainly from Northern Kenya and Somalia but those from other areas were also affected (Bwangamoi, 1969a,b).

According to Bwangamoi (1989) the pathogenesis, gross pathology, histopathology and diagnosis are similar to that of bovine besnoitiosis. This was concluded after observing chronic cases of caprine besnoitiosis and no experimental studies were done to confirm these assertions.

Caprine besnoitiosis is an economically important disease which causes unthriftyness in both adults and kids, predisposes goats to pneumonia, causes sterility in bucks and leads to condemnation of meat and skins (Bwangamoi, 1989). From the foregoing, it is evident that a study on caprine besnoitiosis is necesary in order to understand this important disease. This work was done to study:

1. The level of endemicity of besnoitiosis in domestic ruminants in Kenya.
2. The natural caprine and bovine besnoitiosis in Kenya.
3. The pathogenesis of caprine besnoitiosis.
4. The comparison and contrast of the aetiological agent of caprine besnoitiosis with B. besnoiti in terms of host range, transmission and ultrastructure.
5. Cross-transmission between caprine besnoitia and B. besnoiti.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Aetiology

2.1.1 Classification

Besnoitia besnoiti was discovered in cattle in Southern France by Besnoit and Robin (1912) and was named Sarcocystis besnoiti by Marotel (1912). Since then it has been renamed severally being refered to as Besnoitia besnoiti (Marotel, 1912) by Henry (1913) after he created the genus Besnoitia: Gastrocytis besnoiti (Marotel, 1912) by Brumpt (1913); Globidium besnoiti (Marotel, 1912) by Noller (1920); Globidium leukerti by Henry and Masson (1922) and Bennett (1927).

Babudieri (1932) revised the classification of Sarcosporidia and renamed the organism Besnoitia besnoiti (Marotel, 1912). This has remained to date.

Different researchers have placed the genus Besnoitia in different families. Frenkel (1974) placed it in the family toxoplasmatinae (Biocca, 1956), while Levine (1977) placed it in the family Eimeridae. Frenkel (1977) reclassified the cyst-forming isosporoid coccidia into the family sarcocystidae with two distinct subfamilies namely sarcocystinae (Poche, 1913) and toxoplasmatinae (Biocca, 1956).

Since then this classification has been adopted by Smith (1981) and Soulsby (1982) and will be adopted for this study:

Phylum : Apicomplexa, Levine, 1970

Class : Sporozoea, Leuckart, 1879

Subclass : Coccidia, Leuckart, 1879

Order : Eucoccidiidae, Leger and Dubosq, 1910

Suborder : Eimeriina, Leger, 1911

Family : Sarcocystidae, Poche, 1913

Subfamily : Toxoplasmatinae, Biocca, 1956

Genus : Besnoitia, Henry, 1913

Species : Besnoitia besnoiti (Marotel, 1912) Henry, 1913, Besnoitia darlingi (Brumpt, 1913), Besnoitia tarandi (Hadwen, 1922), Besnoitia benneti (Babudieri, 1932), Besnoitia jellisoni (Frenkel, 1955), Besnoitia wallacei (Frenkel, 1977).

The Besnoitia species that cause besnoitiosis in goats in Kenya and Iran have not been identified (Bigalke, 1981), although it was referred to as Besnoitia besnoiti (Bwangamoi, 1967, 1989; Cheema and Toofanian, 1979).

2.1.2 Morphology

Endozoites and cystozoites of B. besnoiti are crescent to pyriform in shape and measure 5.9 x 2.3 µm and 8.4 x 1.9µm respectively on light microscopy (Bigalke, 1981). Cystozoites scrapped from the conjuctival cysts are either stumpy, measuring 6.2 x 3.1µm or banana shaped, measuring 7.7 x 1.5µm with the nucleus always eccentrically placed on the broad side of the cystozoite (Sannusi, 1991).

The ultrastructure of B. besnoiti has been described by Neuman (1974), Gobel, et. al. (1985) and

Shkap, et. al. (1988), who found that B. besnoiti was typical apicomplexa with a polar ring, conoid, microtubules, rhoptries and micronemes. Heydorn et.al. (1984) described similar features for a Besnoitia species found in the eyes of goats in Kenya. Thus their ultrastructure resembled that observed in related organisms; B. jellisoni (Sheffield, 1966), Toxoplasma gondii (Ogina and Yoneda, 1966; Sheffield and Melton, 1968; Aikawa, Komato, Asai and Midorikawa, 1977). Eimeria tenella (Ryley, 1969) and Eimeria bovis (Sheffield and Hammond, 1966).

It was proposed that differences in ultrastructure features of the different sporozoas be used as the chief criterion for distinguishing them (Honigberg, Balamuth, Bovee, Corliess, Gojdics, Hall, Kudo, Levine, Loeblich, Weiser and Wenrich, 1964; Garnham, 1969, and Levine, 1969, a,b).

2.1.3 Host range

Different Besnoitia species infect different ranges of hosts (Frankel, 1977). B. Wallacei infects rats and mice, while B. jellisoni infects mice, rats and hamsters (Frenkel, 1955). B. besnoiti infects cattle but has been transmitted successfully to rabbits (Pols, 1960, Bigalke, 1968), white mice and gerbils (Neuman, 1962b), sheep and goats (Pols, 1960) and hamsters (Bigalke, 1968). B. darlingi naturally infects lizards and opossums but has also been transmitted to laboratory mice (Schneider, 1965).

According to Marquardt (1970), host and site specificity of sporozoas is useful in differentiating them. Bwangamoi, et. al. (1989) reported that cattle reared together with chronically infected goats for a period of two years did not develop besnoitiosis.

2.1.4 Geographical distribution

Different besnoitia species are distributed in different parts of the world. B. wallacei is found in Hawaii; B. besnoiti, Europe, Asia, Africa and South America; B. jellisoni, North America; B. darlingi, Central America; B. benetti, Africa and B. tarandi, North America (Frenkel, 1977). Lately B. Wallacei have been isolated in Kenya (Ng'ang'a, Munyua and Kanyari, 1994).

The Besnoitia species that affects goats has been reported in Kenya (Bwangamoi, 1967, 1989; Heydorn, et.al., 1984; Bwangamoi, et. al., 1989) and Iran (Cheema and Toofanian, 1979). However, as observed by Bigalke, (1981), the endemicity of the disease in Kenya and Iran is unknown.

2.1.5 Transmission

The life cycle of B. wallacei involves ingestion of mice tissues containing cysts by cats. The cat in turn releases sporulated oocysts in their faeces that are then eaten by mice thus completing the life cycle (Wallace and Frenkel, 1975).

Peteshev et. al. (1974), in yet an unconfirmed report found that domestic and wild cats (Felis lybica) shed oocysts of B. besnoiti in their faeces after being fed on tissues containing the cyst. Based on this report, it was assumed that cattle got infected by ingesting mature isosporan type oocysts shed in the faeces of members of the cat family (Bigalke, 1981).

Healthy cattle naturally got infected when reared with chronically sick ones. Biting flies have also been implicated (Bigalke, 1968). Experimentally, stable flies (Stomoxys calcitrans), mosquitoes (Culex simpsoni), tsetse flies (Glossina brevipalpis) and tabanids (Tabanocella denticornis) were able to mechanically transmit Besnoitiosis from chronically sick cattle, to healthy cattle, rabbits and hamsters (Bigalke, 1968).

Cystozoites inoculated intranasally and endozoites given orally to cattle caused infection. However, cystozoites given orally did not produce disease. Subcutaneous and intraperitoneal inoculation in rabbits and hamsters with either endozoites or cystozoites resulted in clinical besnoitiosis (Bigalke, 1968). Intravenous inoculation of rabbits with cystozoites resulted in acute besnoitiosis (Pols, 1954a; Bigalke, 1967).

Heydorn et.al., (1984) stated that the mode of transmission of Besnoitia in goats remains unclear. Bwangamoi (1989) also reported that transmission of the goat Besnoitia was unclear but speculated that they got

infected by eating sporulated oocyst shed by mongoose in areas of the recent epidemic.

2.1.6 Pathogenesis and pathology

After cattle ingest isosporan type oocysts shed in the faeces of cats, sporozoites emerge and enter circulation and multiply by endodyogeny in endothelial cells of blood vessels. Merozoite-like endozoites (tachyzoites) emerge from the damaged cells to re-invade adjacent or more distant cells to produce further endozoites. This cycle is superseded by cyst formation which commences one week after the initial cycle of proliferation. At this time activated hypertrophic cystozoite containing histiocytic cells, in or in close association with blood vessel walls are observed in the same sites where the endozoites were found (Basson, et. al., 1970).

The merozoite-like cystozoites (bradyzoites) multiply by endodyogeny (Rommel, 1978) in vacuoles of the markedly enlarging host cells with hyperplastic and hypertrophic nuclei to form the characteristically thick walled cysts (Basson, et al, 1970).

The proliferation of endozoites in blood vessels causes degenerative and necrotic vasculitis and thrombosis in medium and small sized blood vessels leading to increased permeability of blood thus resulting to anasarca. The thrombosis and blockage of blood vessels by cysts cause ischaemic infarcts and areas of necrosis in the dermis of the skin, subcutis, nasal

mucosa, larynx, trachea and testis (Basson, et. al. 1970).

The presence of millions of rapidly growing thick walled cysts especially in the dermal papillae and other parts of the stratum papillae and the granulomatus reaction and accompanying fibrosis, hyperkeratosis and acanthosis leads to scleroderma (Basson, et. al. 1970).

According to Cheema and Toofanian (1979), Bwangamoi, et. al. (1989) and Bwangamoi (1989), the chronic clinical disease in goats has similar pathogenesis and pathology to that of cattle described above. However, while the disease description by Basson et. al. (1970) is based on sequential experimental findings, those of Cheema and Toofanian (1979), Bwangamoi, et. al. (1989) and Bwangamoi (1989) are based on findings on natural chronic cases. Thus the early sequence and signs of the disease in goats is unknown.

CHAPTER 3

MATERIALS AND METHODS

3.0 Study of endemicity of Besnoitiosis in domestic ruminants in Kenya

3.1 Survey

Twenty nine districts, representing all the provinces of Kenya i.e. Coast, North Eastern, Eastern, Central, Nairobi, Rift Valley, Nyanza and Western were visited. With the help of the government field veterinary staff, cattle, sheep and goats were examined for cysts in the eye (Bigalke and Neude, 1962), in the individual farms, breeding stations, markets, dips and watering points. Livestock traders were questioned on the origin of their animals. All the findings including diagnosis, sex, breed and age were recorded.

To confirm diagnosis, skin biopsies were aseptically taken from the lateral aspect of the thighs after anaesthetising the area with 2 percent lignocaine hydrochloride. The biopsies were then placed in 10 per cent formalin for at least 48 hours. They were then sectioned, mounted and stained with desired stains as described in the Manual of Histologic and Staining Methods of Armed Forces Institute of Pathology (3rd Edition, 1968, Washington D.C., U.S.A.). Haematoxylin and Eosin (H. & E.) was the stain that was mostly used.

The stained sections were then observed using a light microscope and the findings recorded. Analysis of all the data was done using analysis of variance (ANOVA) as described by Steel and Torrie (1980).

3.2 Field studies on natural caprine and bovine besnoitiosis

3.2.1 Clinical examination of goats and cattle

The three goat breeding stations of Marimanti, Matuga and Bachuma were visited to assess the disease situation in the herds. Each goat was sexed and examined for fever, external parasites, oedema, hyperkeratosis, alopecia, vaginal, nasal and ocular discharges, presence of conjuctival cysts and body condition. Palpation of the testis was done in bucks to check for pain, swelling and indurations.

A group of 30 chronically sick goats were taken to the Veterinary hospital at Kabete and in addition to the above clinical examination, the number of cysts in each eye was counted using a magnifying hand lense and recorded on arrival. External parasites were identified directly if they were ticks. Skin scrappings and nodule discharges from animals with visible skin lesions suspected to have mites or fungus were taken and placed on a clean microscope slide. A few drops of 10 percent Potassium hydroxide was added to digest hair and debris for 20 minutes and the wet slides examined under the light microscope and findings recorded. Where necessary micrographs were taken.

Individual animals in a beef herd at Agricultural Development Corporation farm at Galana suffering from besnoitiosis were examined. The protocol used was similar to that of goats.

3.2.1.2 *Seminology on naturally infected bucks*

Ten bucks were electroejaculated using ITT-Cannon and Pulsator II (Lane Manufacturing Inc., Denver, Colorado, U.S.A.) after clinical examination. The semen was collected in graduated, insulated glass tubes. The volume, consistency, colour, estimated density, wave motion of swirl, individual percentage motility, percentage live, pH, defects and presence of other cells were determined as described by Roberts (1986).

3.3 Study of natural caprine besnoitiosis in confinement

3.3.1 Clinical examination

In addition to the field studies, thirty chronically sick goats, fifteen from Marimanti and fifteen from Bachuma were studied in the Veterinary hospital at Kabete. They were part of 60 chronically sick goats donated by the Government for besnoitia research in the Faculty of Veterinary Medicine, Kabete.

On arrival the animals were dipped in formamidine HCL (Welcare ®, Welcome, Kenya Ltd.) and divided into five groups of six animals each. They were fed on hay and supplemented with wheat bran (Unga, Kenya Ltd.).

The number of cysts in the scleroconjuctiva were counted and the total number of cysts per eye recorded.

The goats were examined daily for any clinical changes. Those that died or were euthanised were fully necropsied. The pathological changes and relative densities of cysts in different tissues were recorded.

3.3.2 Gross pathology

The goats that were euthanised or died naturally and a chronically sick cow that was killed in Galana ADC, were necropsied using standard procedures. The body condition was classifed either as good, fair or poor depending on the muscle and fat cover. Presence and distribution of alopecia and hyperkeratosis were noted. The presence of besnoitia cysts in the different tissues and their relative densities was observed and recorded. Other lesions that were observed were recorded.

3.3.3 Histopathology

Selected tissues were taken from all organs and treated as described in section 3.1 above. The density of cyst distribution in the different tissues was classified either as very heavy, heavy, moderate, scanty or abscent, depending on the number of cysts counted on five microsope fields at a magnification of x40. Those with 80 or more cysts per field were classified as very heavy; 40 to 79 heavy; 5 to 39 moderate and 1 to 5 scanty.

3.3.4 Staining characteristics

Serial sections of a teat of a heavily parasitised doe were made and stained with haematoxylin eosin (H.&E.) Van Gieson's (VG), Periodic Acid Schiff (PAS), Giemsa, Toluidine blue (Tb), RNA/DNA pyronin, Gram and Feulgen stains as described by Humason (1961). They were then

observed under a light microscope, findings recorded and where necessary micrographs taken.

3.3.5 Morphometry of cysts and cystozoites

The sizes of the cysts and cyst walls were measured with an ocular micrometer on the histopathological sections and these were also recorded. 10 cysts were counted per tissue section and their mean diameters and cyst wall thickness recorded as one measurement.

To measure the sizes of the cystozoites, fresh cysts in fascia were disrupted using a pestle and mortar to release them. They were suspended in chilled (+4 °C) phosphate buffered saline (PBS) at pH 7.2. A drop of the cystozoite containing PBS was then placed on a chilled microscope slide and a cover-slip applied. The sizes of 140 cystozoites were measured using a Leitz ocular micrometer at magnification x400 and the average size calculated.

3.3.6 Bacteriology

Fresh lung tissues were aseptically taken from all the goats that died of or were found with pneumonia after euthanasia and submitted for bacteriology. Bacterial isolation and identification was done as described by Merchant and Parker (1967).

3.4 Experimental infections of laboratory and domestic animals with caprine besnoitia

3.4.1 Infective Agent

Chronically infected goats donated by the Government from the Embu, Meru, Isiolo (EMI) and Bachuma goat breeding stations of Eastern and Coast provinces of Kenya, respectively and a chronically infected cow donated by the Agricultural Development Corporation (ADC) farm Galana, in the Tana River district of Coast Province of Kenya acted as the source of infective material. When necessary one or two of these goats were euthenised using pentobarbitone sodium (Sagatal ^{R}) and fascia excised aseptically from the subcutis. The donated cow was killed and fascia removed aseptically from the subcutis.

The excised fascia was then cut into small pieces and pounded using a sterile pestle and mortar to release the cystozoites. To suspend the cystozoites, cold (+4 °C) phosphate buffered saline (PBS) containing 2000 iu procaine penicillin, 2000 mg streptomycin and 40 mg fungizone per millilitre was used. The suspension was then strained through six layers of sterile surgical gauze to remove the fascia debris. A well mixed drop of the strained suspension was placed on a cleaned slide with coverslip and observed under a microscope at magnification x400. Banana shaped motile cystozoites were always present.

The suspensions were then centrifuged at 2000 r.p.m. for 10 minutes and the supernatants decanted. Almost pure pellets of cystozoites were then resuspended in

fresh PBS. Using a haemocytometer the final concentration of cystozoite were adjusted to approximately 1 x 10^9 organisms per millilitre. These were used as the infective materials in all the experiments unless otherwise specified.

3.4.2 Experimental animals (rabbits, rodents, ruminants)

All rabbits, guinea pigs, rats and mice were bought from established colonies at the Veterinary Investigation Laboratories Kabete, while Golden Hamsters were bought from a colony at the Kenya Medical Research Institute (KEMRI). They were brought to the Faculty of Veterinary Medicine, Kabete where they were placed in cages.

The rabbits and guinea pigs were 2 months old while the rats,mice and hamsters were 1 month old. The rabbits were bought tagged but the others were identified using picric acid marks. They were all fed on commercial rabbit and mice pellets (Unga, Kenya Limited). In addition rabbits and guinea pigs were supplemented with cleaned kale leaves.

The goats and bulls were bought from well managed farms in Kiambu district of Central Kenya. They were all examined at the farms and only the healthy animals were bought and brought to the Faculty of Veterinary Medicine, Kabete.

The goats were on average 1-2 years old. Half of them were Galla while the other half were the small East African goats. The bulls were 6 months to 2.5 years old,

half of which were Friesians and the other half Guernseys.

All the ruminants were ear tagged for identification and dewormed with albendazole (Valbazen ® - Kenya Swiss Co. Ltd.). They were fed on hay and supplemented with wheat bran (Unga - Kenya Ltd) and a mineral lick (Maclick - Super ® -Welcome Kenya Ltd.). Water was provided ad-libitum. All the animals were allowed 2 weeks to adapt to the new environment before taking the baseline values. This procedure of acquiring and handling experimental animals was used throughout the period of study unless otherwise stated.

3.4.3 Inoculation protocol used with the experimental animals

Sixteen each of rabbits, rats, mice, hamsters, guinea pigs, goats and eight bulls were used in this experiment. The inoculation protocol used is shown in Table 1.

3.4.4 Clinical examination

Clinical examinations of all the animals were done early in the morning starting from the next day after inoculation. The animals were checked for demeanour, appetite, discharges, oedema, skin changes, abortions, and presence of cysts in the eyes and in external nares of rabbits and rodents.

The body temperature was taken in rabbits and ruminants and recorded in degrees celcius. In addition

Table 1: Inoculation Protocol Used with the Experimental Animals in Experiment One

Animals species	Identification number and sex	Route of inoculation	Number of cystomites administered (x10)	Identification numb and sex of control
Rabbit	201F	SC	4.0	350F 709F 730M
	203F	SC	4.0	350F 709F 730M
	204F	IV	4.0	350F 709F 730M
	205 F	IV	4.0	350F 709F 730M
	212 M	SC	4.0	350F 709F 730M
	810 M	SC	4.0	350F 709F 730M
	820 M	IV	4.0	350F 709F 730M
	836 M	IV	4.0	350F 709F 730M
	884 M	IP	4.0	350F 709F 730M
	900 M	IP	4.0	350F 709F 730M
Rats	01 F	SC	3.0	04F 07F 013M 01
	02 F	SC	3.0	04F 07F 013M 01
	03 F	IV	3.0	04F 07F 013M 01
	05 F	IV	3.0	04F 07F 013M 01
	06 F	IP	3.0	04F 07F 013M 01
	08 F	IP	3.0	04F 07F 013M 01
	09 M	SC	3.0	04F 07F 013M 01
	010 M	SC	3.0	04F 07F 013M 01
	011 M	IV	3.0	04F 07F 013M 01
	012 M	IV	3.0	04F 07F 013M 01
	014 M	IP	3.0	04F 07F 013M 01
	015 M	IP	3.0	04F 07F 013M 01
Mice	02 F	SC	2.5	01F 08F 011M 01
	03 F	SC	2.5	01F 08F 011M 01
	04 F	SC	2.5	01F 08F 011M 01
	05 F	IP	2.5	01F 08F 011M 01
	06 F	IP	2.5	01F 08F 011M 01
	07 F	IP	2.5	01F 08F 011M 01
	09 M	SC	2.5	01F 08F 011M 01
	010 M	SC	2.5	01F 08F 011M 01
	012 M	SC	2.5	01F 08F 011M 01
	014 M	IP	2.5	01F 08F 011M 01
	015 M	IP	2.5	01F 08F 011M 01
	016 M	IP	2.5	01F 08F 011M 01
Hamsters	01 F	SC	3.0	03F 04F 09M 013
	02 F	SC	3.0	03F 04F 09M 013
	05 F	SC	3.0	03F 04F 09M 013
	06 F	IP	3.0	03F 04F 09M 013
	07 F	IP	3.0	03F 04F 09M 013
	08 F	IP	3.0	03F 04F 09M 013
	010 M	SC	3.0	03F 04F 09M 013
	011 M	SC	3.0	03F 04F 09M 013
	012 M	SC	3.0	03F 04F 09M 013
	014 M	IP	3.0	03F 04F 09M 013
	015 M	IP	3.0	03F 04F 09M 013
	016 M	IP	3.0	03F 04F 09M 013
Guinea pigs	02 F	SC	4.0	01F 05F 09M 016
	03 F	SC	4.0	01F 05F 09M 016
	04 F	SC	4.0	01F 05F 09M 016
	06 F	IP	4.0	01F 05F 09M 016
	07 F	IP	4.0	01F 05F 09M 016
	08 F	IP	4.0	01F 05F 09M 016
	010 M	SC	4.0	01F 05F 09M 016
	011 M	SC	4.0	01F 05F 09M 016
	012 M	SC	4.0	01F 05F 09M 016
	013 M	IP	4.0	01F 05F 09M 016
	014 M	IP	4.0	01F 05F 09M 016
	015 M	IP	4.0	01F 05F 09M 016

contd/.....

Animal species	Identification Number and sex	Breed	Route of Inoculation	Number of cystozoites administered	Identification number sex and breed of controls
Goat	226 F	SEA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	241 F	SEA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	242 F	SEA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	244 M	SEA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	245 M	SEA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	256 M	SEA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	259 F	GALLA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	260 F	GALLA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	266 F	GALLA	SC	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	218 M	GALLA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	288 M	GALLA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
	1121 M	GALLA	IV	8.0	240F(SEA) 243M(SEA) 285F(GALLA) 29
Cattle	216 M	GURNSEY		20.0	215M(GURNSEY) 217M(FRESIAN) 220
	218 M	FRESIAN		20.0	215M(GURNSEY) 217M(FRESIAN) 220
	219 M	FRESIAN		20.0	215M(GURNSEY) 217M(FRESIAN) 220
	221 M	GURNSEY		20.0	215M(GURNSEY) 217M(FRESIAN) 220

3.4.5 Samples taken for analysis

3.4.5.1 *Blood*

Blood was collected on alternate days beginning from the next day post-inoculation, after clinical examination in the morning. The rodents and rabbits were bled as described by Weir (1986) while ruminants were bled aseptically from the jugular vein using a syringe and an 18 gauge needle. In all cases blood was placed in bijoul bottles containing EDTA. Haematology was done as described by Schalm (1965).

3.4.5.2 *Buffy coat smears*

Blood in a capillary tube was spun at 2000g for 5 minutes. Using a diamond pen the capillary tube was broken between the buffy coat and the red blood cells. The buffy coat was then spread thinly on a clean microscope slide and stained as described for differential white cell counts above. The slides were then studied using a light microscope and the findings recorded.

3.4.5.3 *Peritoneal smears*

These were taken in rodents only. The animals were placed in an ether containing dessicator until they died. 5 mls of PBS was injected aseptically into the peritoneal cavity using a needle and a syringe. The abdominal cavity was then massaged for two minutes.

The abdominal wall was then opened carefully to avoid leakage of blood into the peritoneal cavity and the

PBS drawn back to the syringe. It was then placed into a centrifuge tube and spun at 2000 g for 10 minutes. The supernatant was decanted and the almost pure pellet of peritoneal cells resuspended in a small amount of PBS.

Slides were made from the resuspended peritoneal contents, stained with Giemsa and observed for presence of bradyzoites.

3.4.5.4 *Tissues for histopathology*

These were either biopsies or tissues taken during necropsy. The biopsies were taken weekly from the skin of the limbs neck and scrotum of bulls and treated as described in section 3.1. One bull was unilaterally castrated on day 56 and sections of the epididymis, testis, vas deferens and pampiniform plexus were made. Another bull was killed 60 days after inoculation and selected samples of the skin, testis, pampinform plexus, muzzle, trachea and turbinates taken for histopathology.

On days 7, 14, 21, 42 and 56 after inoculation one of each of the rabbits, guinea pigs, hamsters, rats and mice was killed and fully necropsied and tissues were taken from all body organs. Smears were made from the peritoneal fluid as described in section 3.4.5.3.
Four goats, two bucks and two does were euthanised 56 days after inoculation, necropsied and tissues taken for histopathology.

All the tissues taken for histopathology were treated as described in section 3.1.

3.4.5.5 *Tissues for electron microscopy*

These tissues were taken from freshly killed animals or as biopsies measuring 1 x 1 x 1 mm and processed using the following procedures as described by Ito and Karnovsky, (1968):

a) The small tissues were fixed in "Yellow fix" composed of 4 per cent paraformaldehyde dyed with 0.01 per cent trinitrocresol and 2 percent glutaldehyde for 2 hours at +4°C.

b) The tissues were rinsed three times for 5 minutes each using a phosphate buffer of pH 7.2 - 7.4 at +4°C.

c) The tissues were then post-fixed in 1 per cent osmium tetroxide made in phosphate buffer of pH 7.2 -7.4 for 4 hours at +4°C.

d) They were then rinsed three times for 5 minutes each in physiological saline at +4°C.

e) They were then dehydrated through graded concentrations of acetone from 30%, 50%, 70%, 90% and 100% at +4°C.

f) They were then infiltrated with Durcupan ACM (Fluka AG, Switzerland) which is an epoxy resin following the protocol below:

i) 3 parts of acetone to 1 part of Durcupan 1 for 1 hour at room temperature.

ii) 2 parts of acetone to 2 parts of Durcupan 1 for 1 hour at room temperature.

iii) 1 part of acetone to 3 parts of Durcupan 1 for 1 hour at room temperature.

iv) They were then put through two changes of 100 per cent Durcupan 1 for 2 hours at 50°C.

v) The tissues were then put into Durcupan 2 into which a hardener had been added for 2 hours at 50°C.

g) The tissues were then embedded in Durcupan 2 for 48 hours at 80°C.

h) They were then sectioned using an ultramicrotome (Reichert Austria Om u2) using glass knives.

i) The sections were then mounted on copper grids and stained with Uranyl acetate and counter stained with lead citrate.

j) They were then examined using a Carl Zeiss 109 electron microscope and micrographs taken and studied.

3.5 Comparative experimental infectivity studies of Besnoitia besnoiti and caprine besnoitia

Failure to elicit any disease in rabbits, rats, mice, hamsters, guinea pigs and cattle using caprine besnoitia made it necessary to compare its infectivity with B. besnoiti at least in rabbits, cattle and goats.

3.5.1 Infective materials

Caprine besnoitia and *B. besnoiti* were prepared from chronically infected goat and cow respectively as described in section 3.4.1.

3.5.2 Experimental animals

Four rabbits, goats and cattle previously used as controls in the "Experimental infections of laboratory and domestic animals with caprine besnoitia" (see section 3.4.2) were used in this study.

3.5.3 Inoculation protocol

The infection protocol used with the experimental animals is shown in Table 2 below.

Table 2: Inoculation Protocol Used for Inoculation of the Experimental Ruminants in Experiment Two

Animal Species	Identification Number and sex	Route of inoculation
Rabbit	350 F	Subcutenous
	730 M	Subcutenous
	709 F	Subcutenous
	775 M	Subcutenous
Goat	240 F	Subcutenous
	291 M	Subcutenous
	285 F	Subcutenous
	243 M	Subcutenous
Cattle	215	Subcutenous
	217	Subcutenous
	220	Subcutenous
	222	Subcutenous

Number of cystozoites Administered	Besnoitia spp. used
4.0	B. Besnoiti
4.0	B. besnoiti
4.0	Caprine Besnoitia
4.0	Caprine Besnoitia
8.0	B. besnoiti
8.0	Besnoitia besnoitia
8.0	Caprine Besnoitia
8.0	Caprine Besnoitia
20.0	B. besnoiti
20.0	B. besnoiti
20.0	
20.0	Caprine Besnoitia

3.5.4 Clinical examination and sample taking

These were done as described in sections 3.4.4 and 3.4.5 respectively.

3.6 Infection of rabbits and cattle previously exposed to caprine besnoitia with B. besnoiti

The availability of rabbits and bulls previously exposed to caprine besnoitia without developing clinical disease and availability of a field isolate of B. besnoiti made this study imperative.

3.6.1 Infective material

This was prepared as described in section 3.4.1.

3.6.2 Experimental animals

Four rabbits and four bulls were used in this study. Two rabbits Nos. 709F and 775M and two bulls Nos. 220M and 222M previously infected with caprine besnoitia without developing clinical besnoitiosis were used as the test animals. Two fresh rabbits and two bulls were used as controls.

3.6.3 Inoculation protocol

The infection protocol used on the experimental animals is shown on Table 3.

Table 3

The inoculation protocol used with the experimental animals in experiment three:-

Animal Species Used	Identification No.and Sex (F-Female M-Male)	Route of Inoculation	Number of cystozoite Admin. ($X10^9$)	Besnoitia used
Rabbit	709F*	Subcut.	4.0	B.
	775m*	enous	"	besnoiti
	230F	"	"	"
	231M	"	"	"
Cattle	220M*	"	20	"
	222M*	"	"	"
	223M	"	"	"
	224M	"	"	"

* Previously inoculated with caprine Besnoitia in 3.5.

3.6.4 Clinical examination and sample taking

These were done as described in sections 3.4.4 and 3.4.5 respectively.

3.7 Experimental infection routes of goats with caprine besnoitia

3.7.1 Infective Material

3.7.1.1 *Implantation*

Intact fascia with large number of besnoitia cysts was used for implantation.

3.7.1.2 *Needle pricking*

A chronically infected goat with a large number of subcutenous besnoitia cysts was used alive as the source of infective material in needle pricking trials.

3.7.1.3 *Intrauterine*

Two chronically infected pregnant does and two pregnant recently inoculated does were used as source of the infective material for the vertical infection trials.

3.7.1.4 *Intranasal, intraconjuctival, ocular and oral*

The infective material used in these transmission trials was prepared as described in section 3.4.1.

3.7.2 Experimental animals

Fourteen does were bought and prepared as was described in section 3.4.2. Two does that had chronic besnoitiosis were also included. All the does were allowed to co-habit with intact bucks for a period of 1 month before being recruited into the experiment.

3.7.3 Inoculation protocol

3.7.3.1 *Implantation*

A chronically infected goat was euthanised for preparation of infective material as was described in

section 3.4.1. Aseptically, a stab incision was made on the lateral aspect of the neck of two recipient goats, and a pocket made using a pair of scissors through the incision.

Intact fascia was then removed aseptically from the killed chronically infected goat and pushed into the pocket made in the neck of the recipient goats and a single nylon suture placed to close the incision before spraying the wound with a fly repellant containing tetracycline spray.

3.7.3.2 *Needle pricking*

A chronically infected goat acted as the source of the infective material. This goat together with the two recipient goats were sedated with 0.3ml of xylazine hydrochloride (Rompun ^{R} - Bayer). The planter surfaces of the limbs extending from the carpal to the metacarpal joints were shaved with a scalpel blade and disinfected using surgical spirit. The skin was then desensitised by ring blocking using 2 per cent lignocaine hydrochloride at the level of the carpal joint. A sterile 25 gauge needle was roughened at the tip with a sterile file. Using the roughened needle, alternative deep pricks were made into the subcutis of the chronically infected goat then into the subcutis of the recipient animals. This was repeated at least 150 times.

3.7.3.3 *Intrauterine*

Two chronically infected and two healthy goats were allowed to cohabit with the intact buck for 3½ months.

The healthy goats were then inoculated intravenously with approximately 10 x 10^9 cystozoites. All the animals were then monitored daily. On day 24 post-inoculation, two does were euthanised (one chronically sic and the other inoculated 24 days ago) and together with the fetuses fully necropsied. Skin, uterine and placental tissues were taken both from the dam and fetus for histopathology. The others were allowed to carry their pregnancies to term.

3.7.3.4 *Intranasal*

Two healthy does were inoculated with approximately 10 x 10^9 cystozoites in near pellet form PBS one month after cohabiting with intact bucks. The animals were restrained manually and the head held high to allow the deposition of drops of phosphate buffered saline (pH 7.2) containing large number of cystozoites deep into the nasal cavity.

3.7.3.5 *Intraconjuctival*

Approximately 10 x 10^9 cystozoites contained in 2 mls of PBS were injected subconjuctival into two goats. In one goat the whole amount was injected into one eye while in the other the infective material was divided into two and 1ml was injected into each eye.

3.7.3.6 *Ocular*

Drops of PBS containing approximately 10 x 10^9 cystozoites were placed into the dorsal part of the eyes of two goats using a dropper. This was done very slowly to avoid spillage.

3.7.3.7 *Oral*

A large volume of cystozoites (approximately 40 x 10^9) was given orally to two goats using a 20 ml syringe.

3.7.4 Clinical examination

This was done as described in section 3.4.4. All except two of the does used in intrauterine transmission trials were allowed to carry their pregnancies to term or until they aborted naturally.

3.7.5 Samples taken for analysis from infected does

3.7.5.1 *Blood smears*

These were taken daily from all the infected animals and prepared as described for differential white cell counts by Schalm (1965) and examined for bradyzoites.

3.7.5.2 *Tissues for histopathology*

These were either biopsies taken weekly or necropsy tissues from euthanised animals. Both were handled as described in section 3.1.

Euthanasia was done on two goats used in intrauterine transmission trials, one being the chronically sick and the other being the freshly

infected. This was on the fourth month of pregnancy. Tissues were taken during necropsy from both the does and the fetuses and treated like other tissues above.

3.8 Pathogenesis of caprine besnoitiosis

3.8.1 Infective material

The infective material was prepared as described in section 3.4.1.

3.8.2 Experimental animals:

Eighteen goats, (Nos. 282, 283, 286, 289, 290, 292, 293, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306 and 307), all female Small East African breed, were used in this study. They were acquired and treated as described in section 3.4.2.

3.8.3 Inoculation protocol:

All the goats were infected intravenously the same day using 8 ml of inoculate containing approximately 1 x 10^9 cystozoites per ml.

3.8.4 Clinical examination:

This was done daily as described in section 3.4.4.

3.8.5 Samples taken for analysis

3.8.5.1 *Blood smears:*

These were made on alternate days as described by Schalm, (1965), stained with Giemsa and checked for bradyzoites.

3.8.5.2 *Tissues for histopathology*

The goats were serially killed on days 3, 7, 11, 14, 17, 20, 23, 26, 33, 40, 46, 56, 66, 76, 86, 108 and 305 respectively. They were fully necropsied, taking keen observation on presence of cysts in different organs.

Selected tissues were taken for histopathology and prepared as described in 3.1. They were then examined under a microscope and the sizes of cysts measured using an ocular micrometer and these were recorded. Where necessary, micrographs were taken. For the purpose of this study, the muzzle area close to the nostrils was used to study cyst development in all animals to avoid tissue variation.

3.9 Comparative Ultrastructural Studies on *B. besnoiti* and caprine besnoitia

3.9.1 Electron Microscopy Tissues

Fresh subcutis was taken from a just killed bull and doe both with chronic besnoitiosis 70 days post-inoculation and processed as described in section 3.4.5.5. They were then examined and studied using a Carl Zeiss 109 transmission electron microscope, then micrographs taken.

CHAPTER 4

RESULTS

4.0 Study of the endemicity of besnoitiosis in domestic ruminants in Kenya

4.1 Survey

Table 4 shows the besnoitiosis infection rate in goats in the 29 districts visited and 3 whose goats were examined in markets outside the district. The highest infection rate was in Mandela (36.3 percent) followed by Kwale (35.2 percent) Isiolo (34.8 percent), Marsabit (33 percent), Wajir (28.0 percent), Nairobi (25.6 percent), Meru (24.4 percent), Garissa (21.0 percent), Taita Taveta (18.0 percent), Embu (16.7 percent), Kitui (8.6 percent), Machakos (6.5 percent), Laikipia (2.8 percent), Kajiado (1.6 percent), Turkana (0.9 percent) and Elgeyo Marakwet (0.8 percent). In total 37,955 goats were examined.

Examination of individual herds using the cysts in the eye method (Figure 1) in some districts revealed very high infection rates. Rhamu division in Mandela and Kina division in Isiolo had infection rates of 51 percent. The goat breeding stations Marimanti in Meru, Matuga and Bachuma both in Kwale had high infection rates of 46, 36 and 34 percent respectively. Ethiopian and Somali goats examined along River Daua on the North Eastern boundary of Kenya with Ethiopia and Somalia had infection rates ranging from 30 to 51 percent.

Table 4: Infection Rates in Goats Naturally Infected with Besnoitiosis

Province	District	Goats Examined	Predominant Breed(s)	No. of Does	No. of Sick Does	No. of Bucks	No. of sick Bucks	No. of Kids	No. of Sick Kids	Overall Percen Sick Goats
Coast	Lamu	407	Small E.A	252	0	84	0	71	0	0
	Kilifi	384	S.E.A	234	0	78	0	72	0	0
	Tana River	433	Galla	267	0	91	0	75	0	0
	Kwale	1161	S.E.A.	651	275	290	123	220	11	35.2
	Taita Taveta	550	S.E.A.	372	74	102	19	76	6	16
N. Eastern	Mandela	4168	Galla	2498	1078	836	351	834	84	38.3
	Wajir*	289	Galla	105	30	174	51	0	0	28
	Garissa*	167	Galla	56	13	101	22	0	0	21
Eastern	Marsabit*	230	Galla	96	31	134	45	0	0	33
	Isiolo	2821	Galla	1694	693	567	238	560	49	34.8
	Meru	2411	S.E.A Galla Toggenberg	1368	378	582	157	461	23	24.4
	Embu	1831	SEA/GALLA	1091	189	548	106	181	11	16.7
	Kitui	1298	SEA/Galla	756	67	416	156	9	8.6	
	Machakos	2799	SEA/Galla	1710	110	829	52	260	101	6.5
Nairobi	Nairobi	1052	SEA/Galla	394	114	475	155	183	0	25.6
Central	Kiambu	505	SEA/Galla	320	0	98	0	87	0	0
	Murang'a	752	SEA	450	0	150	0	152	0	0
Rift Valley	Kajiado	3208	SEA/Galla	1600	32	1028	19	348	0	1.6
	Narok	2413	SEA	115	0	1028	0	270	0	0
	Nakuru	100	SEA	600	0	284	0	116	0	0
	Baringo	3410	SEA	1991	0	903	0	516	0	0
	Elg. Marakwe	711	SEA	364	4	189	2	158	0	0.8
	West Pokot	2141	SEA	1209	0	'570	362	0	0	
	Turkana	1618	SEA	778	8	709	7	131	0	0.9
	Laikipia	633	SEA/Galla	404	14	129	4	100	0	2.8
Western	Tranzoia	455	SEA	177	0	138	0	140	0	0
	Bungoma	277	SEA	124	0	41	0	62	0	0
	Busia	270	SEA	142	0	47	0	81	0	0
	Vihiga	138	SEA	72	0	25	0	41	0	0
Nyanza	Kisumu	442	SEA/Togenber	279	70	0	93	0	0	
	South Nyanz	388	SEA	198	0	67	0	128	0	0
	Kisii	115	SEA	43	0	22	0	50	0	0

*Districts not visited Animals examined in the markets.

NB: = Small East African.

SEA - Small East African
Togenbe - Toggenberg

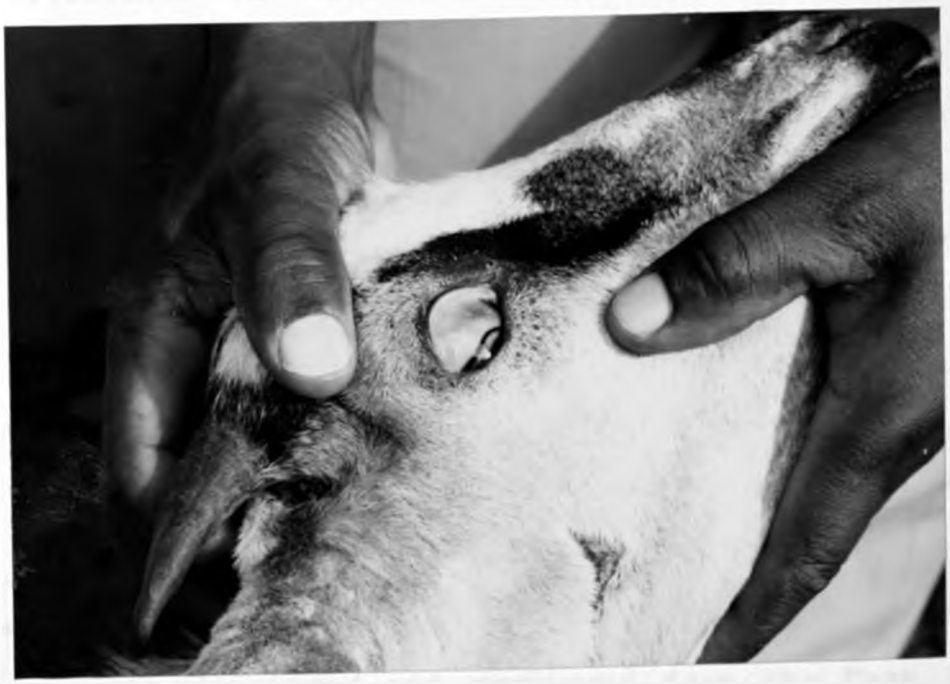


Figure 1

A photograph of a goat suffering from chronic natural besnoitiosis. The head is held with the scleroconjuctiva against the sunlight to expose the many small white spots which represent the besnoitia cysts.

In all the districts where the overall infection rate was above 6.0 percent, kids (2 to 6 months) were affected. However, Nairobi was an exception in that it had an infection rate of 25.6 percent but kids were not affected.

No caprine besnoitiosis was diagnosed in Central, Western and Nyanza provinces. It was also no diagnosed in some districts of the Rift Valley like Baringo, Nakuru and Narok. In the Coast Province the disease was not found in Lamu, Kilifi and Tana River districts.

Statistical analysis did not show significant difference in infection rates of males (mean 18.1 percent) and females (mean 18.4 percent) but kids were significantly less affected than adults (mean 4.2 percent).

A total of 14,818 sheep namely Red Maasai, Dorper and Merino were examined in the 29 districts visited. However none of the sheep was diagnosed as having besnoitiosis. Examination of all skin biopsies did not reveal any besnoitia cysts.

A total of 16,014 cattle were examined in the 29 districts visited during the survey. Bovine besnoitiosis was only diagnosed and confirmed from the Tana River district in the Coast Province where out of 2340 adult animals were examined, 260 i.e. 11.1 percent were found suffering from chronic besnoitiosis.

4.2 Field studies on natural caprine and bovine besnoitiosis

4.2.1 Clinical Observations

A total of 730, 690 and 471 goats were closely examined in the goat breeding stations of Marimanti, Matuga and Bachuma, out of which 339 (46.4 percent), 249 (36 percent) and 160 (34 percent) respectively had conjuctival cysts.

In Marimanti four goats (0.6 percent); comprising three does and one buck had fever of 41.2°C, dullness, anorexia, lacrimation, marked generalised oedema and in the buck the testis were painful to touch. In Matuga nine goats (1.3 percent) comprising of seven does and two bucks had similar clinical signs. No conjuctival cysts were observed in these animals. Clinical pneumonia was diagnosed in 10 percent of the animals in the three herds.

Alopecia and hyperkeratosis were observed in 38 percent of the goats mainly on the face, carpus, hock and brisket. Demodex caprae was diagnosed in six goats, one from Marimanti and five from Bachuma. Sarcoptes scabeii was diagnosed in three goats all from Bachuma.

All the goats from Marimanti and Bachuma had one or both of Amblyomma variegatum and Rhipicephalus evertsi. Both ticks were found around the head, on the underside of the ear, below the tail, around the perineum, the inguinal region, the lower part of the limbs, under the dew claws and between the hooves.

A total of 260 cattle had chronic besnoitiosis with the typical elephant skin disease (Figure 2) with alopecia, hyperkeratosis, fissures, mucopurulent nasal discharge and unthriftness.

4.2.1.2 *Seminology on naturally infected bucks*

The semen characteristics of the ten infected bucks that were electroejaculated are shown in Table 5. The volume ranged from 1.5ml to 3.0ml with a mean of 2.25ml.

The colour of the semen was either yellowish-cream (30%), clear-watery (20%) or milky (50%).

The actual sperm count ranged from 0 to 1.5 million. The wave motion of swirl was absent in the semen of all the bucks.

The percentage individual sperm motility ranged from 0 to 80 percent with a mean of 54.5%. The mass motility ranged from 0 to 80% with a mean of 49.0%. The percentage live sperms ranged from 0 to 85% with a mean of 50.5%.

The pH ranged for 6.6 to 6.8 with a mean of 6.72. Spermatozoa defects were noted in eight of the ten semen samples. The loss of the tail piece and oblique attachment of the middle piece to the head were the most common defects observed.

Table 6: Semen Characteristics of 10 Bucks Suffering from Chronic Natural Desmoitiosis

	1/Mar	2/Mar*	3/Mar	85/Mar	052/Bac	5/Mar.	924/Kibu	741/Kibu	1016/Kib	79/Kibu
Volume (ml)	1.0	0.0	1.5	1.0	0.0	0.0	1.0	0.0	2.0	
Colour	Yellow	Clear-	Milky	Milky	Watery	Milky	Yellow	Milky	Milky	Yellow
Density (Estimate)	2	0	1	2	0	3	3	2	3	3
Concentration (Actual Count)	.5 x 10	0.0 x 10	1 x 10	.8 x 10	0.0 x 10	1 x 10	1 x 10	1 x 10	1.2 x 10	1.5 x 10
% Motility (Individual)	50	0	50	70	0	80	80	60	80	75
Mass Motility	40	0	40	60	0	80	60	50	80	80
% Live	45	0	50	85	0	80	80	70	55	75
pH	6.8	6.8	6.8	6.6	6.8	6.7	6.6	6.7	6.8	6.6
Defects	+	-	+	+	-	+	+	+	+	+

Mar.* - Marimanti

Bach.** - Bachuma

Kibu - Kiburine

The normal values for normal semen characteristics in bucks are volume (0.7-3ml) Colour (thick creamy) estimated concentration density (5) actual sperm count (2.5 x 10 /ml) individual percentage motility (App. 100) Mass motility (80-100%) defects (<20%) live (>80%) and pH (6.8). Roberts, 1986)

Actual counts (millions)

Figure 2[2] A photograph of a cow suffering from chronic natural besnoitiosis. The animal shows the typical elephant disease with alopecia, hyperkeratosis, prominent skin folding and fissures. It also has poor body condition.

4.3 Study of natural caprine besnoitiosis in confinement

4.3.1 Clinical Observations

A summary of the clinical observations on the thirty goats studied in confinement at the animal hospital, Kabete is shown in Table 6. Six goats (20%) had good body condition, eighteen (60%) had fair body condition while the other six (20%) had poor body condition. The goats in good body condition had an average of 18.9 cysts and not more than 27 cysts per eye. Those in fair body condition had an average of 53 cysts but three of them (16.7%) had more than 100 cysts per eye. Those in poor body condition, five (83.3%) had more than 100 cysts and only one had less than 100 cysts per eye.

Alopecia and hyperkeratosis was found in six goats with fair body condition (33.3%) and in six goats with poor body condition (100%). Goats with good body condition had no alopecia or hyperkeratosis.

Alopecia and hyperkeratosis were found in the muzzle, face, neck, brisket, carpus, hock, thigh, scrotum, backline and ears. Among the twelve goats with alopecia and hyperkeratosis seven (66.7%) had them on the muzzle, six (50%) on the face, one (8.3%) on the neck, eleven (91.7%) on the carpus and hock, two (16.7%) on the thigh, three (25%) on the backline and two (16.7%) on the ears. In the three bucks alopecia and hyperkeratosis was observed in the scrotum of all three animals.

Sarcoptes scabeii was observed in two goats that had alopecia and hyperkeratosis in the ears. Demodex caprae was diagnosed in two goats, while Rhipicephalus evertsi

Table 6: Antemortem Findings in Naturally Infected Goats

Animal No.	Sex	Killed or DN	(G) (F) Body Cond.	No. of Cysts in Eyes		Areas of Alopecia and Hyperkeratosis										Other findings			
				R	L	Muzzle	Face	Neck	Brisket	Carpal	Hock	Thigh	Scrotum	Backline	Ear	Ticks	Mange	Pneu.	Diarr.
079	F	DN	Good	20	16	-	-	-	-	-	-	-	-	-	-	+		+	
047	F	DN	Fair	32	48	-	-	-	-	-	-	-	-	-	-	+	-	+	-
194	F	DN	Fair	60	56	-	-	-	+	+	+	-	-	-	-	+	-	+	-
003	F	DN	Poor	>100	>100	+	+	-	+	+	+	-	-	+	-	+	-	+	-
375	F	DN	Fair	11	16	-	-	-	+	-	-	-	-	-	-	+	-	+	-
078	F	DN	Fair	42	46	-	-	-	-	-	-	-	-	-	-	+	-	+	-
177	F	DN	Fair	59	66	-	-	-	-	-	-	-	-	-	-	+	-	+	-
029	F	DN	Poor	>100	>100	+	+	-	-	+	+	-	-	-	+	+	S	+	-
195	F	DN	Good	17	15	-	-	-	-	-	-	-	-	-	-	+	-	+	-
001	F	DN	Fair	90	94	+	+	-	-	+	+	-	-	-	-	+	-	+	
169	F	DN	Poor	>100	>100	+	+	+	+	+	+	-	-	-	-	+	-	+	-
394	M	DN	Poor	40	66	-	-	-	+	+	+	-	+	+	-	+	-	+	
191	F	K	Good	22	14	-	-	-	-	-	-	-	-	-	-	+	-	-	-
543	F	K	Fair	72	69	-	-	-	-	-	-	-	-	-	-	+	D	-	-
175	F	K	Fair	28	40	-	-	-	-	-	-	-	-	-	-	+	-	-	-
071	F	K	Good	16	27	-	-	-	-	-	-	-	-	-	-	+	-	-	-
340	M	K	Poor	>100	>100	+	+	-	-	+	+	+	+	-	-	+	D	-	-
215	F	K	Fair	53	65	-	+	-	-	+	+	-	-	-	+	+	S	-	-
239	F	K	Fair	36	34	-	-	-	-	-	-	-	-	-	-	+	-	-	-
147	F	K	Good	12	17	-	-	-	-	-	-	-	-	-	-	+	-	-	-
182	F	K	Fair	46	52	-	-	-	-	-	-	-	-	-	-	+	-	-	-
721	F	K	Poor	>100	>100	-	-	-	-	+	+	-	-	-	-	+	-	-	-
199	F	K	Fair	21	20	-	-	-	-	-	-	-	-	-	-	+	-	-	-
109	F	K	>100	>100	>100	+	+	-	+	+	+	+	-	-	-	+	-	-	-
216	F	K	Fair	46	46	-	-	-	-	-	-	-	-	-	-	+	-	-	-
077	F	K	Fair	35	44	-	-	-	-	-	-	-	-	-	-	+	-	-	-
011	F	K	Good	22	25	-	-	-	-	-	-	-	-	-	-	+	-	-	-
205	F	K	Fair	>100	>100	-	-	-	-	-	-	-	-	-	-	+	-	-	-
052	M	K	Fair	>100	>100	+	+	-	-	+	+	-	+	-	-	-	-	-	-
507	F	K	Fair	31	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NB:

DN - Died naturally
K - Killed
G - Good
F - Fair
Pneu. - Pneumonia
Diarr. - Diarrhoea
S - Sarcoptic mange
D - Demodectic mange

and Amblyomma variegatum were observed in the thirty goats. The animals were free from helminths when the feaces were examined for strongyle eggs in the laboratory.

4.3.2 Gross pathology at postmortem

In addition to the above antemortem findings, large numbers of besnoitia cysts were found in different tissues on postmortem.

The highest concentration of cysts were found in the skin, subcutis and intermuscular fascia of muscles in the regions of hock-colium, carpal-colium, backline, stifle-hock, humerus-radioulnar, and hip-stifle (Figure 3). Other areas with equally high concentration of cysts were the teats, face, superficial blood vessels of the face, and lower limbs, tunica albuginea, and pampiniform plexus. The turbinates and trachea were also in some cases found to have a very high concentration of cysts.

Large numbers of cysts were also observed in the ligaments and tendons of the lower limbs. The periosteum of the metatarsal, metacarpal and phalanges were heavily parasitised. The surface of the joint capsules of the hock, carpus, metatarsus, metacarpus and phalanges were also heavily parasitised.

The distribution of cysts in the respiratory system was inconsistent. There were moderate to scanty numbers in the nostrils. In some animals large number of cysts were observed in the turbinates, nasal septum, larynx and trachea, while in others only a moderate number were

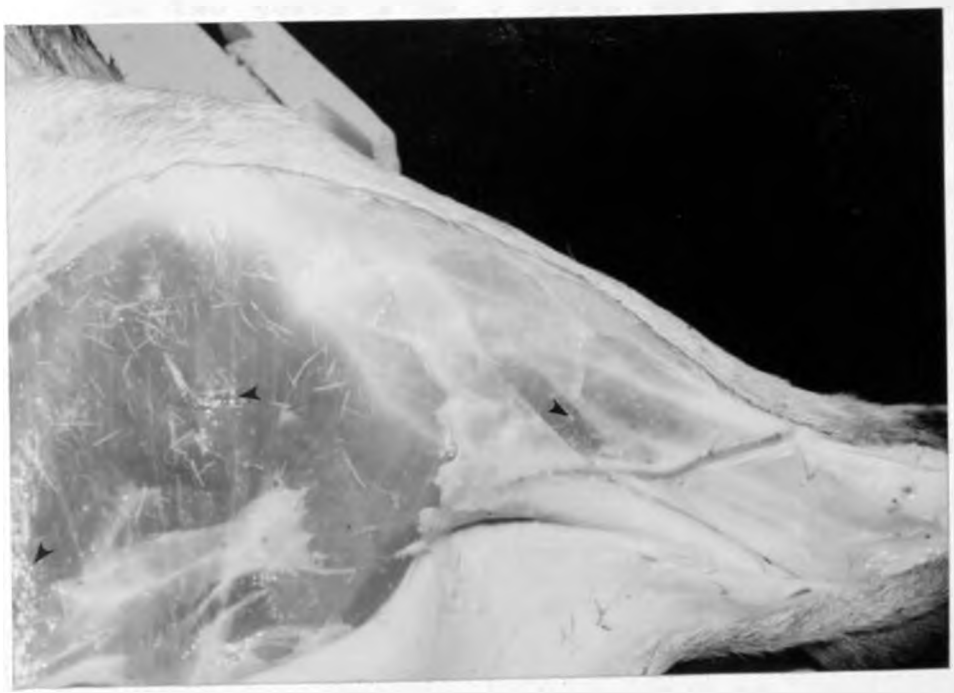


Figure 3
§ A photograph showing a flayed lateral aspect of thigh of a goat suffering from chronic natural besnoitiosis. The many white spots on the transluscent fascia (arrows) are the besnoitia cysts.

observed while yet in others none were seen. In one case large numbers of cysts were observed in the epiglottis and larynx of an animal that could not bleat.

In the cardiovascular system two cysts were seen under the heart valves of only two goats. No cysts were seen in the coronary vessels, myocardium or pericardium. Few cysts were observed in the carotid blood vessels of one goat. Blood vessels within the thorax, abdomen and pelvis did not have cysts, except for the last part of the testicular artery just before it forms part of the pampiniform plexus.

In two goats 2 to 3 cysts were observed in the jugular and the vena cava in the neck region. At the bifurcation of the dorsal aorta of two goats, few cysts were observed. The number of cysts however increased in all goats from the iliac and brachial arteries peripherally until the digital blood vessels were almost occluded by polypoid projections of the cysts which were markedly more at the bifurcations. Thrombosis was observed in both digital arteries and veins of fore and hind limbs.

The alimentary system had few cysts. A moderate to scanty number of cysts were observed in the buccae, gums, soft and hard palates, tongue, pharynx and anus. No cysts were observed in the oesophagus, forestomachs, intestines, liver, gall bladder and mesenteric blood vessels.

In the urogenital system only the teats, tunica albuginea and pampiniform plexus were heavily

parasitised. But increased numbers of cysts was observed in the cervix, vagina and vulva. No cysts were found in the ovaries, uterus and oviducts. A moderate number of cysts were observed in the mammary gland. In the bucks, a large number of cysts were found in the pampiniform plexus, in the vas deferens and epididymis. Few cysts were found in the testis. In one heavily parasitised buck a blackish-brown pigment was observed in the tunica vaginalis (Figure 4). Very few cysts were found in the urethra and penis in bucks. No cysts were observed in the accessory glands. In both sexes no cysts were seen in the kidneys, ureters and bladder.

In the reticulo-endothelial systems cysts were found on the capsule of the precrural, inguinal and prescapular lymph nodes but not in their parenchyma. Internal lymph nodes and the thyroid gland had no cysts.

There were very few cysts on the dura matter of the spinal cord but other parts of the central nervous system were spared.

Four goats died one week after arrival in Kabete while the remaining eight died during the six months that the animals were under observation. These animals developed fever of 40.5 - 41°C, mucopurulent nasal discharges, anorexia and then died. One of the goats was found with pneumonia after euthanasia. Gross pathology revealed pneumonia with froth in the air passages.

Figure 4

A photograph of two testis of a buck suffering from natural chronic besnoitiosis. The testis are atrophied and their parenchyma degenerated and yellow in colour. The tunica vaginalis (tv) has dark brown hemosiderin pigment (H). Many white spots (arrows) representing besnoitia cysts are visible on the intact fascia (f), tunica vaginalis, pampiniform plexus (P) and cranial epididymis (e).

4.3.3 Histopathology

Histopathology confirmed the distribution densities of cysts observed grossly in the skin and subcutis; intermuscular fascia; alimentary, urogenital, cardiovascular and respiratory systems as shown on Tables 7,8,9,10,11,12 respectively.

Deep seated tissues had few cysts while the superficial ones had many cysts. However some superficial tissues which were poorly vascularised e.g. ears and tail also had few cysts.

The cysts were found in the endothelium of blood vessels where they caused varying degrees of occlusion. Some of the cysts were also found in the muscular layer of the blood vessels and many were found outside the blood vessels.

The cysts comprised of a parasitophorous vacuole that contained cystozoites. The vacuole was surrounded by a thin primary (inner) cyst wall. This was in turn surrounded by the host cell that was multinucleate, then a thick, broad, collagenenous non-cellular, pale staining secondary (outer) cyst wall. To the outside of the outer cyst wall was intact cells of the host connective tissue. The host cell which forms the parasitophorous vacuole of the cyst had either round nucleus in young cysts or highly flattened ones in old mature cysts. In some cysts septae formation was observed.

Majority of the cysts did not elicit a tissue reaction. However, some especially the degenerating ones

Table 7: Distribution of Besnoitia Cysts on Examination of Skin and Subcutis of Naturally Infected Goats

No.	Muzzl	Lips	Face	Cheek	ID	Neck	Briske	Shoul	H.R.U.	Ears	C.C	Thora	Abd.	Glute	H. S.	S. H.	H. C	Scr.	Prepu	Tail	B.L.	Per.
079	++	++	+++	+	++	++	+	++	+++	+	+++	++	+	++	+++	+++	+++	0	0	+	++	+
047	++	++	+++	+	++	++	+	++	+++	+	+++	++	+	++	++	+++	+++	0	0	+	+++	+
194	+++	++	+++	++	++	++	++	+++	+++	+	++++	++	+	++	+++	+++	++++	0	0	+	+++	0
003	+++	++	++++	++	+++	+++	++	++++	++++	+	++++	+++	++	+++	+++	++++	++++	0	0	+	++++	++
375	+	+	++	+	+	++	++	++	++	+	++	++	+	+	++	+++	+++	0	0	+	++	+
078	++	++	+++	+	++	++	+	++	+++	+	+++	+	+	+	+++	+++	+++	0	0	+	+++	+
177	+++	++	+++	+	++	++	++	+++	+++	+	+++	+++	++	+++	+++	+++	+++	0	0	+	+++	+
029	++++	+++	++++	++	++	+++	+++	+++	+++	+	++++	+++	+++	+++	+++	++++	++++	0	0	+	++++	+
195	++	++	+++	+	++	++	+	++	+++	+	+++	++	+	++	++	+++	+++	0	0	+	++	+
001	+++	++	+++	++	++	++	+++	+++	+++	+	++++	+++	++	+++	++	++++	++++	0	0	+	+++	+
169	++++	++	++++	+++	+++	+++	++++	+++	+++	+	++++	++++	++	+++	+++	+++	++++	0	0	+	+++	+
334	+++	++	+++	+	++	++	++	+++	+++	+	++++	+++	++	++	+++	+++	++++	++++	++	+	+++	+
191	+	+	++	+	+	+	+	+	++	+	+++	++	+	+	+++	++	+++	0	0	+	++	+
543	++	++	+++	++	++	+++	++	++	+++	+	+++	++	+	++	++	+++	+++	0	0	+	+++	+
175	++	++	+++	+	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	+++	+
071	+	+	++	+	+	++	++	++	++	+	+++	+	+	+	++	++	+++	0	0	+	++	+
340	++	+++	++++	++	+++	++++	++	++++	++++	+	++++	+++	+++	+++	++++	++++	++++	++++	++	+	+++	+
215	++	++	+++	+	++	++	++	+++	+++	+	+++	++	++	++	+++	+++	++++	0	0	+	++++	+
239	++	++	+++	+	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	++	+
147	+	+	++	+	+	+	+	+	+	+	++	+	+	+	++	++	++	0	0	+	++	+
162	++	++	+++	+	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	++	+
721	+++	++	++++	+++	+++	+++	++	+++	++++	+	++++	++	+++	+++	++++	++++	++++	0	0	+	+++	+
199	+	+	++	+	+	+	+	++	++	+	++	+	+	+	++	++	++	0	0	+	++	+
109	+++	++	++++	+++	+++	+++	+++	+++	+++	+	++++	+++	+++	++++	++++	++++	++++	0	0	+	++++	++
218	++	++	+++	+	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	++	+
077	++	++	+++	++	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	++	+
011	+	++	++	+	++	+	+	++	++	+	++	+	+	+	++	++	++	0	0	+	++	+
205	+++	+++	++++	+++	+++	+++	++	+++	++++	+	++++	+++	++++	+++	++++	++++	++++	0	0	+	++++	+
052	+++	++++	++++	+++	++	+++	++	+++	++++	+	++++	+++	+++	+++	++++	++++	++++	++++	++	+	++++	+
507	++	++	+++	+	++	++	++	++	+++	+	+++	++	+	++	+++	++	+++	0	0	+	++	+

N.B. From Table 7-12

++++ - Very heavy

+++ - Heavy

++ - Moderate

+ - Scanty

0 - None

— - Not examined

Index: Muz. - Muzzle, ID - Intermandibular, Briske - Brisket, Shoul - Shoulder, H.R.U. - Humerus radial-ulna, C.C. - Carpal colum, Thora - Thorax, Abd. - Abdomen, Glute - Gluteus, H.S. - Hip stifle, S.H. - Stifle hock, H.C. - Hock Colium, Scr. - Scrotum, Prepu - Prepuce, B.L. - Backline Per. - Perineum

Table 8: Distribution of Besnoitia Cysts on Examination of the Intermuscular Fascia in the Muscolo-Skeletal System of Naturaly Infected Goats

No.	Brachio cephalicu	Sterno- mandibulars	Trapezi	Del- todeus	Supra spinatus	Biceps	Tricep	Pectora	Cute. Tru.	Ex. Ob. Abd.	Lat. Dor.	Long Dor.	Linia Alba	Ser.	In. Ob.	Vastus Lateralis	STM	M.G.	G.B.	M.A.M.
079	++	++	++	++	++	++	++	++	++	+	++	+++	+	++	+	+++	++	++	+++	++++
047	++	++	++	++	++	++	++	++	++	+	++	+++	+	++	+	+++	+++	+++	+++	++++
194	++	++	++	+++	+++	+++	++	+++	+++	++	++	+++	+	++	+	++	++	++	++	+++
003	++	+++	++	++++	++++	++++	+++	+++	++	+	+++	++++	+	++	+	++++	++++	+++	++++	++++
375	++	+	+	++	++	++	++	++	+	+	++	+++	+	++	0	+++	++	++	+++	++++
078	++	++	++	++	++	++	++	++	+	+	++	+++	+	++	+	+++	++	++	+++	++++
177	++	++	++	+++	++	+++	+++	+++	++	++	++	+++	+	++	+	+++	++	++	+++	++++
029	+++	++	++	+++	+++	+++	+++	+++	+++	++	++	+++	+	++	+	+++	++	++	+++	++++
195	++	++	++	++	++	++	++	++	++	++	++	+++	+	++	+	+++	++	++	+++	+++
001	++	++	++	+++	+++	++	++	+++	+++	++	++	+++	+	++	+	+++	++	++	+++	++++
169	+++	+++	++	+++	++	+++	++	+++	+++	++	++	++++	+	++	+	++++	++	+++	++++	++++
334	++	++	++	+++	+++	++	++	+++	+++	++	++	+++	+	++	+	+++	++	++	+++	++++
191	++	+	+	+	++	++	++	++	++	++	++	+++	+	++	+	+++	++	++	+++	++++
543	+++	++	++	++	++	++	++	++	++	+	+	+++	+	+	+	+++	+++	+++	+++	++++
175	++	++	++	++	++	++	++	++	+	+	++	+++	+	++	+	+++	++	++	+++	++++
071	++	++	++	++	++	++	++	++	+	+	+	+++	+	+	+	+++	++	++	+++	++++
340	+++	++	++	++++	++++	++++	+++	++	++	+	+++	++++	+	+++	+	++++	+++	+++	++++	++++
215	++	++	++	+++	+++	+++	++	++	++	+	++	++++	+	++	+	++++	+++	+++	++++	++++
239	++	++	++	++	++	++	++	++	++	+	++	+++	+	++	+	+++	++	++	+++	+++
147	+	+	+	+	+	+	+	+	++	+	+	+++	+	+	0	+++	++	++	+++	+++
162	++	++	++	++	++	++	+	++	++	++	++	+++	+	++	+	+++	++	++	+++	++++
721	+++	+++	+++	+++	+++	+	++	++	++	++	++	++++	+	++	+	++++	+++	++	++++	++++
199	+	+	+	++	++	++	++	++	++	+	+	+++	+	+	+	+++	++	++	+++	++++
109	+++	+++	+++	+++	+++	+++	++	+++	++	++	+++	++++	+	+++	+	++++	+++	+++	++++	++++
218	++	++	++	++	++	++	++	++	++	+	++	+++	+	++	0	+++	++	++	+++	++++
077	++	++	++	++	++	++	++	++	++	++	++	+++	+	++	+	+++	++	++	+++	++++
011	++	++	++	++	++	++	++	++	++	++	+	+++	+	+	+	+++	++	++	+++	+++
205	+++	++	++	+++	+++	+++	+++	+++	+++	++	++	++++	+	++	+	++++	+++	+++	++++	++++
052	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	++++	+	+++	+	+++	++	++	+++	++++
507	+	+	+	++	++	++	++	+	++	+	++	++++	+	++	++	+++	++	++	+++	++++

Index: Cute. Tru. - Cutenous Trunkii, Ex. Ob. Abd. - External oblique abdominis, Lat. Dor. - Latismus dorsi, Ser. - Serratus, In.Ob. - Internal oblique - abdominis, STM - Semitendinosus semimembranous, M.G. - Medial gluteus, G.B. - Gluteus biceps, M.A.M. - Muscles of antebrachium and manus/leg and foot

++++ - Very heavy, +++ - Heavy, ++ - Moderate, + - Scanty, 0 - None
— - Not examined

Table 9: Distribution of Besnoitia Cysts on Examination
System of Naturally Infected Goats

No.	Bucca	Gums	H.P.	S.P.	Tongu	Gland(s)	Phary	Tonsil	Oeso.	Fore.
079	+	+	0	+	+	0	0	0	0	0
047	+	+	0	+	+	0	0	0	0	0
194	0	+	0	+	+	0	0	0	0	0
003	++	++	0	++	++	0	++	0	0	0
375	0	+	0	+	+	0	0	0	0	0
078	+	+	0	+	+	0	0	0	0	0
177	+	+	0	+	+	0	0	0	0	0
029	++	++	+	++	+	0	++	0	0	0
195	0	+	0	+	+	0	0	0	0	0
001	+	+	0	+	+	0	0	0	0	0
169	+	+	0	+	+	+	+	0	0	0
334	+	+	0	+	+	0	0	0	0	0
191	+	+	0	+	+	0	0	0	0	0
543	+	+	0	+	+	0	+	0	0	0
175	+	+	0	+	+	0	0	0	0	0
071	+	+	0	+	+	0	0	0	0	0
340	++	++	0	+	+	+	++	0	0	0
215	+	+	0	+	+	0	0	0	0	0
239	+	+	0	+	+	0	0	0	0	0
147	+	+	0	+	+	0	0	0	0	0
182	+	+	0	+	+	0	0	0	0	0
721	++	++	+	+	+	+	++	0	0	0
199	0	0	0	+	0	0	0	0	0	0
109	++	++	0	+	++	+	++	0	0	0
218	+	+	0	+	+	0	0	0	0	0
077	+	+	0	+	+	0	0	0	0	0
011	+	+	0	+	+	0	+	0	0	0
205	+	+	0	+	+	0	0	0	0	0
052	+	+	0	+	+	0	+	0	0	+
507	+	+	0	+	+	0	0	0	0	0

N.B. From Table 7-12

++++ - Very heavy
+++ - Heavy
++ - Moderate
+ - Scanty
0 - None
— - Not examined

Index: H.P. - Hard palate, S.P. - Soft palate,
Oeso. - Oesophagus, Fore. - Forestomachs,
L.I. - Large intestine, Rectu. - Rectum,

Abo	S.I	L.I	Rectu	Anus	Liver	G.B	Pan
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	0	0	0
0	0	0	0	+	+	0	0
0	0	0	0	+	0	0	0

Tongu - Tongue, Phary - Phayrnx,
, Abo. - Abomasum, S.I. - Small intestine,
G.B. - Gall bladder, Pan. - Pancrease

Table 10: Distribution of Besnoitia Cysts on Examination of the Urogenital System of Naturally Infected Goats

No.	K. & Ur.	Bl.	P.	P. & S.	T.P.	T.V.	T.A.	Epi.	V.D.	P.P.	Ov./Ovi.	Uteru	Cervix	Vagin	Vulva	M. G.	Teats
079	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	++	+++
047	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	++	+++
194	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	++	++++
003	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	++	++++
375	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	++++
078	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	+++
177	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	+	++++
029	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	++++
195	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	++	+++
001	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	++++
169	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	+	++++
334	0	0	+	0	+	+++	+++	++	+	++++	-	-	-	-	-	-	-
191	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	+	+++
543	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	+++
175	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	++	+++
071	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	++	+++
340	0	0	+	0	+	+++	+++	++	+	++++	-	-	-	-	-	-	-
215	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	+++
239	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	+	+++
147	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	+	++
162	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	++	+++
721	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	++++
199	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	+	+++
109	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	++	++++
218	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	++	+++
077	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	++	++
011	0	0	-	-	-	-	-	-	-	-	0	0	0	+	+	++	+++
205	0	0	-	-	-	-	-	-	-	-	0	0	+	+	++	+	++++
052	0	0	++	0	+	++++	++++	++	+	++++	-	-	-	-	-	-	-
507	0	0	-	-	-	-	-	-	-	-	0	0	+	+	+	+	+++

N.B. From Table 7-12

++++ - Very heavy
+++ - Heavy
++ - Moderate
+ - Scanty
0 - None
— - Not examined

;Index: K. & ur. - Kidney and Ureters, Bl. - Bladder, P. - Penis, P. & S. - Prostate and seminal vesicle, T.P. - Testicular parenchyma, T.V. - Tunica vaginalis, T.A. - Tunica albigenia, Epi. - Epididymis, V.D. - Vas deferens

Table 11: Distribution of Besnoitia Cysts on Examination of the Cardio Vascular System on Naturally Infected Goats

No.	H.& C.	P.A./V.	Caroti C.	Re.	Tr. F.	M. & Ma. La. Ar./Ve.	Ra. Ar.	Ra. Ar./Ve	Di.Ar./Ve.	In. & Ex. Il.	Fe.	Sapheno	Ti. Ar.	Di. Ar.	Te. Ar.	Pu. Ar	In. Ar.	G. & M.	Vena Cava	Jugula	Dorsal Aorta
079	0	0	0	0	+	+++	+	+	++	+	+	+++	+++	+++	-	-	+	0	0	+	0
047	0	0	0	0	+	+++	+	+	++	+	+	++	++	+++	-	-	+	0	0	0	0
194	0	0	0	0	+	++++	+	+++	++++	+	++	++++	+++	+++	-	-	+	0	0	0	0
003	0	0	0	0	+	+++	+	+++	++++	+	+	++++	+++	+++	-	-	++	0	0	+	0
375	0	0	0	0	+	+++	+	+	+++	+	+	++	++	+++	-	-	+	0	0	0	0
078	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
177	0	0	0	0	+	+++	+	+++	++++	+	++	++++	++++	++++	-	-	++	0	0	+	0
029	0	0	0	0	+	+++	+	+	++++	+	+	+++	+++	++++	-	-	++	0	0	0	0
195	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
001	0	0	0	0	+	+++	+	+	++++	+	+	+++	+++	++	-	-	+	0	0	0	0
169	0	0	0	0	+	++++	+	++	++++	+	++	+++	+++	+++	-	-	++	0	0	+	0
334	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	+++	+	+	0	0	0	0
191	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
543	0	0	0	0	+	+++	+	+	+++	+	+	++	++	+++	-	-	+	0	0	+	0
175	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
071	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
340	+	0	0	0	+	++++	+	++	++++	+	++	+++	+++	+++	+++	+	++	0	+	+	0
215	0	0	0	0	+	+++	+	+	+++	+	+	+++	+++	+++	-	-	+	0	0	0	0
239	0	0	0	0	+	+++	+	+	+	+	+	+++	+++	+++	-	-	+	0	0	0	0
147	0	0	0	0	0	+++	+	+	+++	+	++	+++	+++	+++	-	-	+	0	0	+	0
162	0	0	0	0	+	+++	+	+	+++	+	++	+++	+++	+++	-	-	+	0	0	0	0
721	0	0	0	0	0	++++	+	++	++++	+	++	++	++	+++	-	-	+	0	0	+	0
199	0	0	0	0	+	+++	+	+	+	+	+	+++	++	+++	-	-	+	0	0	0	0
109	0	0	0	0	+	++++	+	+	+++	+	++	+++	+++	+++	-	-	+	0	0	+	0
218	0	0	0	0	+	+++	+	+	+++	+	++	+++	+++	+++	-	-	+	0	0	0	0
077	0	0	0	0	+	+++	+	+	++	+	+	++	++	+++	-	-	+	0	0	0	0
011	0	0	0	0	0	+++	+	+	+	+	+	+++	+++	+++	-	-	+	0	0	0	0
205	0	0	0	0	+	+++	+	+	++++	+	++	++	++	+++	-	-	+	0	0	+	0
052	+	0	+	0	+	++++	+	++++	++++	++	+	+++	++++	+++	++++	++++	++	++	0	+	+
507	0	0	0	0	+	+++	+	+++	++	+	+	+++	+++	++++	-	-	+	0	0	0	0

N.B. From Table 7-12

++++ - Very heavy
+++ - Heavy
++ - Moderate
+ - Scanty
0 - None
— - Not examined

Index: H.&C. - Heart & corony vessels, P.A./V. - Pulmonary artery/vein, C.Re. - Cerebral rete, Tr. F. - Transverse facial, M. & Ma. La. Ar./Ve. Maxillary & mandibular labial artery/vein, Ra. Ar. - Radial artery, Ra. - Radial vein, Di.Ar. - Digital artery, In.&Ex.Il. - Internal & external iliacs, Fe. - Femoral, Sa. - Salphenous, Ti. - Tibial, Div. - Digital veins, Te.Ar. - Testicular Artery, Pu.Ar. - Pudendal artery, In. Ar. - Intercostal artery, G. & M. - Gastric and mesenteric

Table 12: Distribution Densities of Besnoitia Cysts on Examination of the Respiratory System of Naturally Infected Goats

No.	Nostrils	Nasal	Turbinates	Epiglottis	Larynx	Trachea	Brochi	Brochioles	Lung	Diaphram	Intercos Muscles
079	++	0	0	0	-	0	0	0	0	0	+
047	+	0	++	+	+	+	+	0	0	0	+
194	+	0	0	+	-	0	0	0	0	0	+
003	++	+	++++	++	+++	+++	++	+	0	0	+
375	+	0	0	0	0	0	0	0	0	0	+
078	++	+	+	+	+	0	+	0	0	0	+
177	+++	++	0	0	0	0	0	0	0	0	+
029	++	0	++++	++	+++	+++	+	0	0	0	+
185	++	+	0	+	0	0	0	0	0	0	+
001	++	+	0	+	0	0	0	0	0	0	+
169	++	+	0	++	+	0	0	0	0	0	+
334	++	++	0	0	0	0	0	0	0	0	+
191	+	0	0	0	0	0	0	0	0	0	+
543	++	0	++	++	++	++	+	0	0	0	+
175	+	+	++++	++	++	++	+	+	0	0	+
071	+	0	-	-	0	0	0	0	0	0	+
340	++	+	+++	++++	++++	+++	+	0	0	0	+
215	++	+	0	0	0	0	0	0	0	0	+
239	+	0	0	0	0	0	0	0	0	0	+
147	++	+	+++	++	++	++	-	0	0	0	+
162	++	+	0	0	0	0	0	0	0	0	+
712	++	+	0	0	0	0	0	0	0	0	+
199	+	0	+++	++	++	++	+	0	0	0	+
109	++	+	+	+	+	0	0	0	0	0	+
218	+	+	+++	++	++	++	+	+	0	0	+
077	++	+	0	-	0	0	0	0	0	0	+
011	+	+	++	++	++	++	+	0	0	0	+
205	++	+	0	0	0	0	0	0	0	0	+
052	++	+	++++	+	+++	+++	+	+	0	0	+
507	+	0	0	0	0	0	0	0	0	0	+

N.B. From Table 7-12

++++ - Very heavy
+++ - Heavy
++ - Moderate
+ - Scanty
0 - None
— - Not examined

Index: Trache - Trachea, Intercos - Intercostal

without an intact cyst wall, provoked severe tissue reaction, with infiltration of large numbers of polymorphonuclear cells.

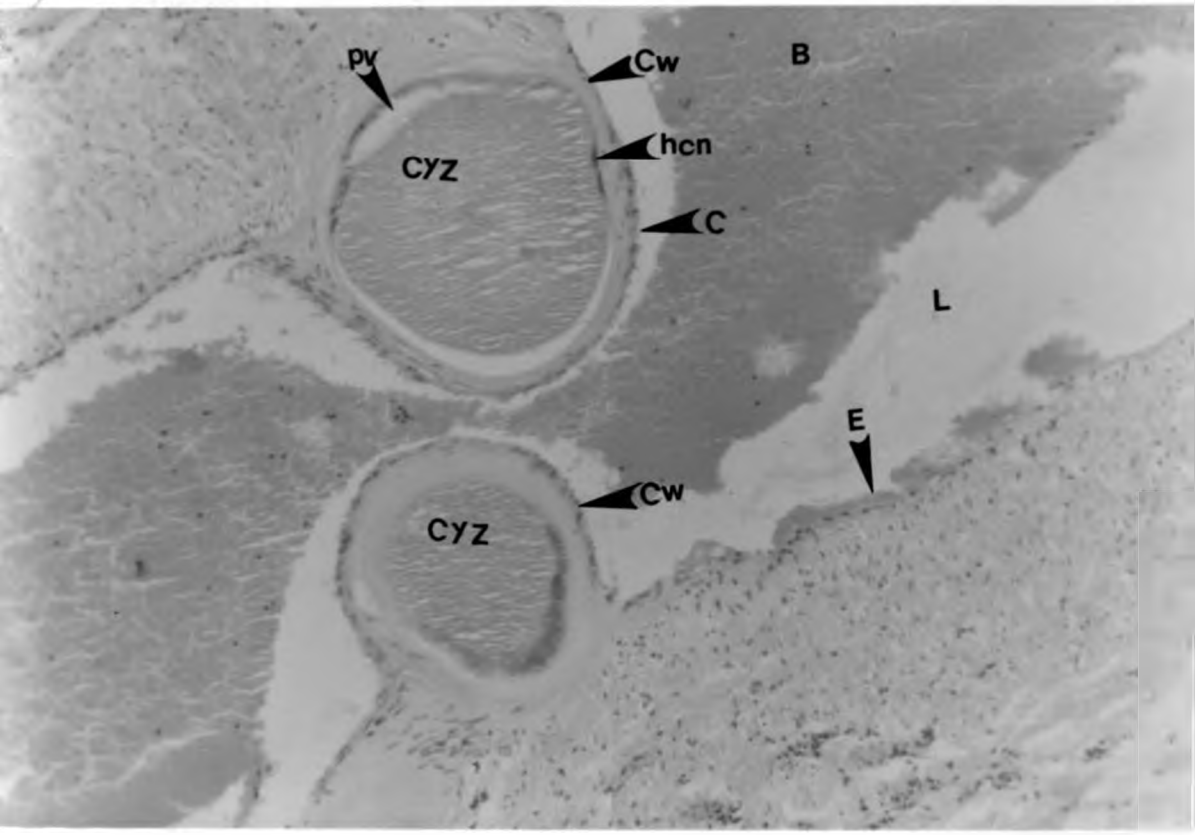
In the bucks many cysts were found in the epididymis, tunica albuginea and pampiniform plexus. In the pampiniform plexus the cysts greatly occluded the lumen of the blood vessels. Few cysts were found in the parenchyma of the testis. The parenchyma was degenerated, atrophied, and with no signs of spermatogenesis (Figures 5 & 6).

4.3.4 Staining characteristics of caprine besnoitia cysts from a goat suffering from natural chronic besnoitiosis

Haematoxylin and Eosin (H.& E.) stained cystozoites pink and this was more intense towards the periphery where it tended to be purple. The cystozoites appeared like strands of beads packed closely on top of each other.

The inner cyst wall stained pink-purple and was thin. The outer cyst wall was thick and stained light pink and tended to merge with host tissues. The host cell nuclei appeared dark tan (brown) and were either round in young cysts, oval in medium aged cysts or completely flattened in old cysts.

In some cysts septae formation was observed dividing the cysts into equal daughter cysts and the septae line originating from nuclei at the poles of the cysts.



[5] Figure 5

A micrograph showing a longitudinal section of a digital arteriole with two intact besnoitia cysts (C) growing on the endothelium (E) of the blood vessel. The cysts have intact cyst walls (CW) and the highly granular cystozoites (CYZ) inside the parasitophorous vacuole (PV). The host cell nucleus (hcn) which is almost fully flattened is visible in the upper cyst. The cysts have not elicited any inflammatory reaction around them but are almost occluding the lumen (L) thus reducing blood (B) flow (H & E x 200).

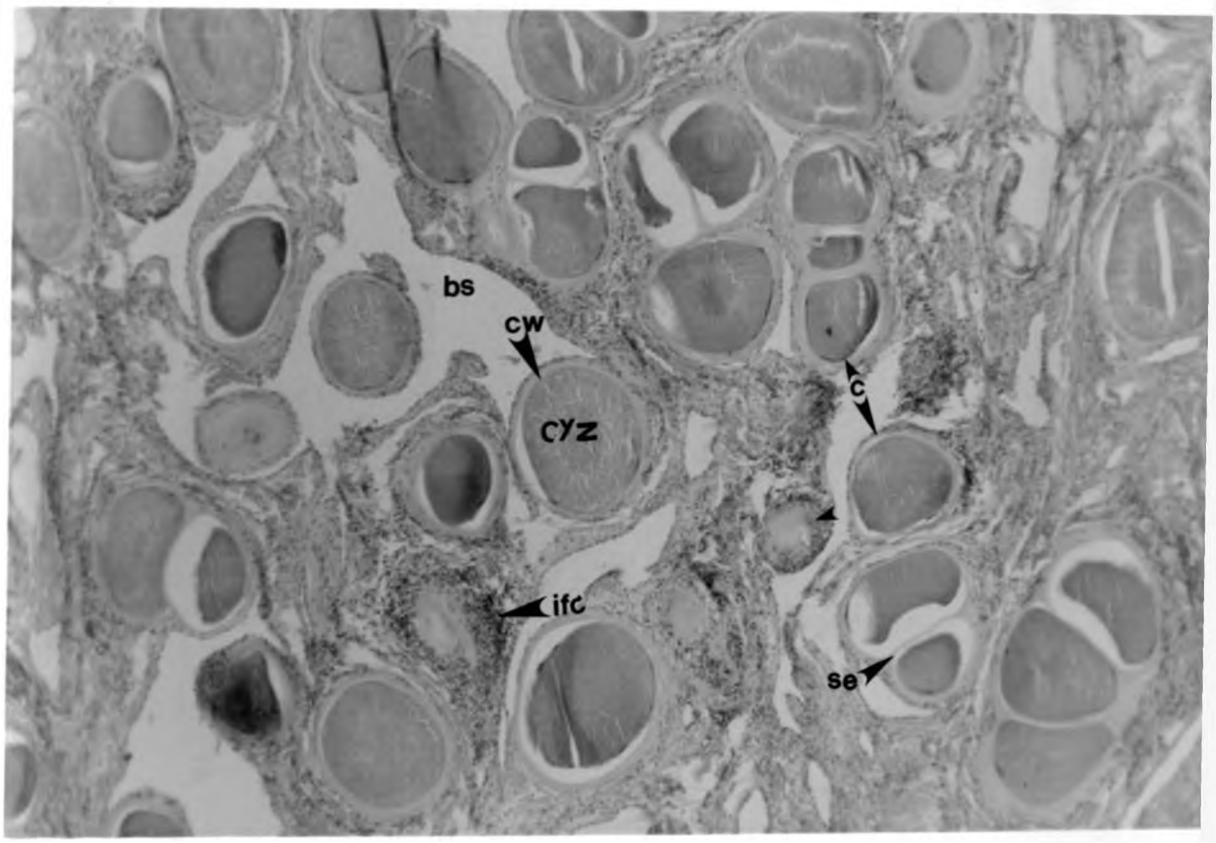


Figure 6

A micrograph showing a transverse section of the pampiniform plexus of a buck suffering from natural chronic besnoitiosis. There are many intact besnoitia cysts (C), with cystozoites (CYZ) and intact cyst wall (CW). Some have septae (se) dividing them. Some cysts are degenerative (dc and arrow) and their cyst walls are not intact. They have elicited severe inflammatory with massive infiltration of inflammatory cells (ifc). Most of the blood sinuses (bs) have been occluded by the cysts (H&e x 200).

The septae line also was found to originate from a homogenous mass that stained with differing intensity, with the centre staining light pink like the cystozoites and the periphery purplish like the inner wall. The septae could also be seen in some cysts with nuclei in it.

In some old cysts degeneration was observed and the cystozoites appeared homogenously purplish (Figure 7).

Giemsa stained the cystozoites blackish and this was more intense on the periphery than the centre. The inner cyst wall stained black-brown to crimson like the cystozoites while the outer wall stained crimson and merged with host tissues. The host cell nuclei stained crimson like the outer wall but appeared granular. The homogenous mass described above had blackish centre and blue-black periphery (Figure 8).

Cystozoites were gram negative and stained light pink to maroon. They appeared granula and those on the periphery stained much deeper than the ones on the centre in old cysts. In young cysts the cystozoites stained uniformly. The inner cyst wall stained maroon like the cystozoites and was very clear. The outer cyst wall stained maroon then red and purplish red as it merges with host tissues (Figure 9).

The host cell nucleus stained homogenously maroon and were difficult to delienate. Septae formation was clear but the homogenous mass was difficult to discern (Figure 9).

Van Gieson (VG) stained cystozoites yellow to dark-brown. Peripheral cystozoites stained dark brown while the middle cystozoites stained yellow. Individual cystozoites stained dark-brown at the centre and yellow on the periphery. The inner cyst wall stained dark-brown especially in young cysts while the outer wall stained light to intense red. The host cell nucleus stained dark-brown to black. The homogenous mass seen in some cysts stained yellow at the centre and dark brown on the periphery. Septae formation was quite clear (Figure 10).

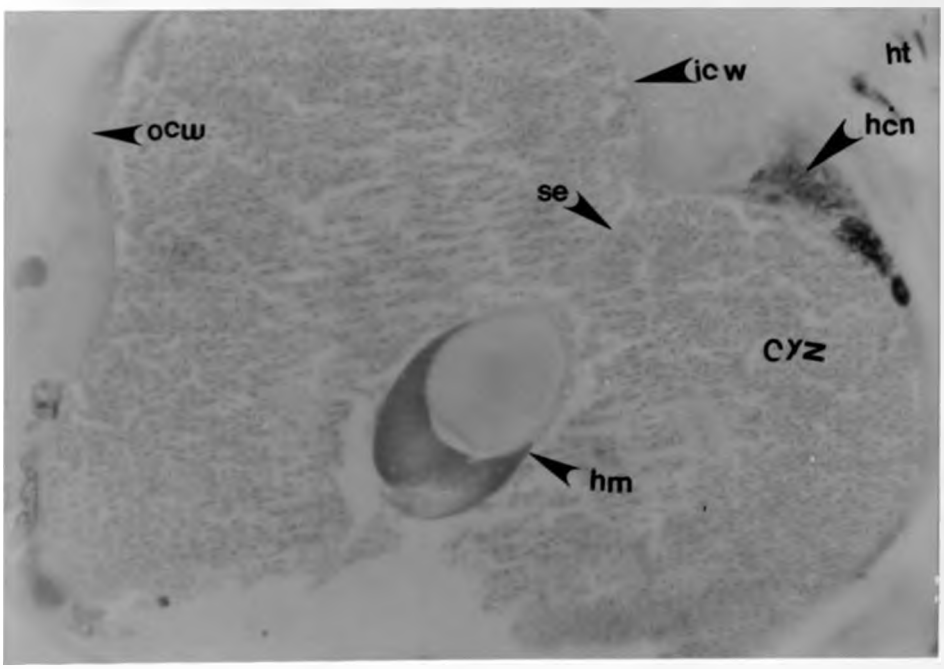


Figure 7

A micrograph showing part of a cyst with the highly granular cystozoites (CYZ) which are deep staining on the periphery compared to the centre. A homogenous mass (hm) with a reddish brown periphery and a light pink area can also be seen. The hm is connected to the thin inner cyst wall (icw) with a septae (se) line. The icw stains similarly to the cystozoites while the thick lighter staining outer cyst wall (ocw) merges with the host tissues (ht). The dark tan (brown) host cell nucleus (hcn) are also present. (H&E x 400).

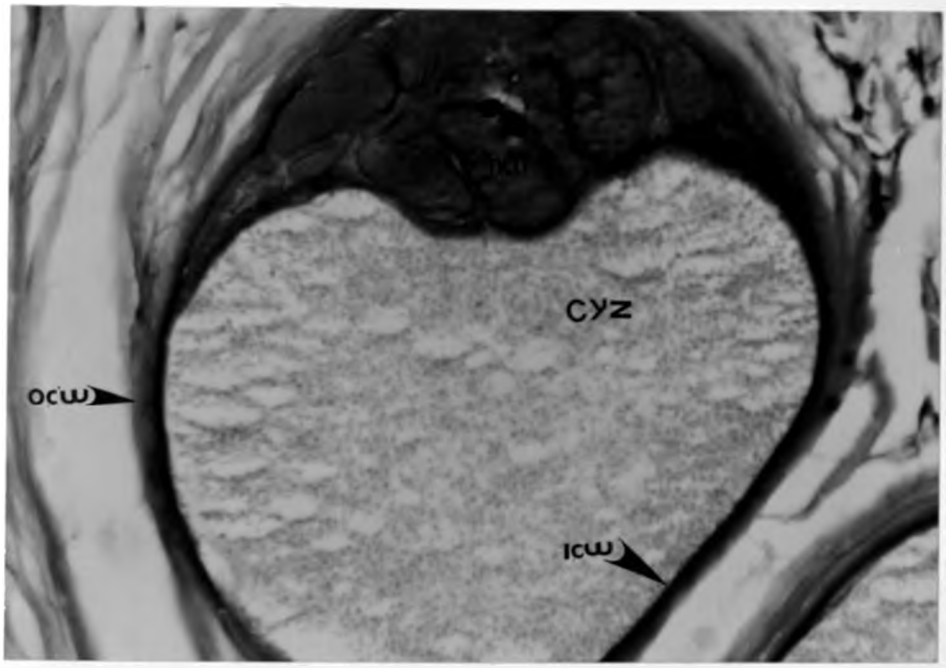


Figure 8

A micrograph showing a section of a cyst with the blackish staining highly granular cystozoites (cyz) which are deep staining on the periphery and light in the centre. The thin inner cyst wall (icw) stains similarly to the cystozoites while the thick outer cyst wall (ocw) stains crimson. The hyperplastic, multinucleate host cell nuclei (hcn) which stain similar to the outer cyst wall can be seen between the icw and the ocw. (Giemsa x 400).

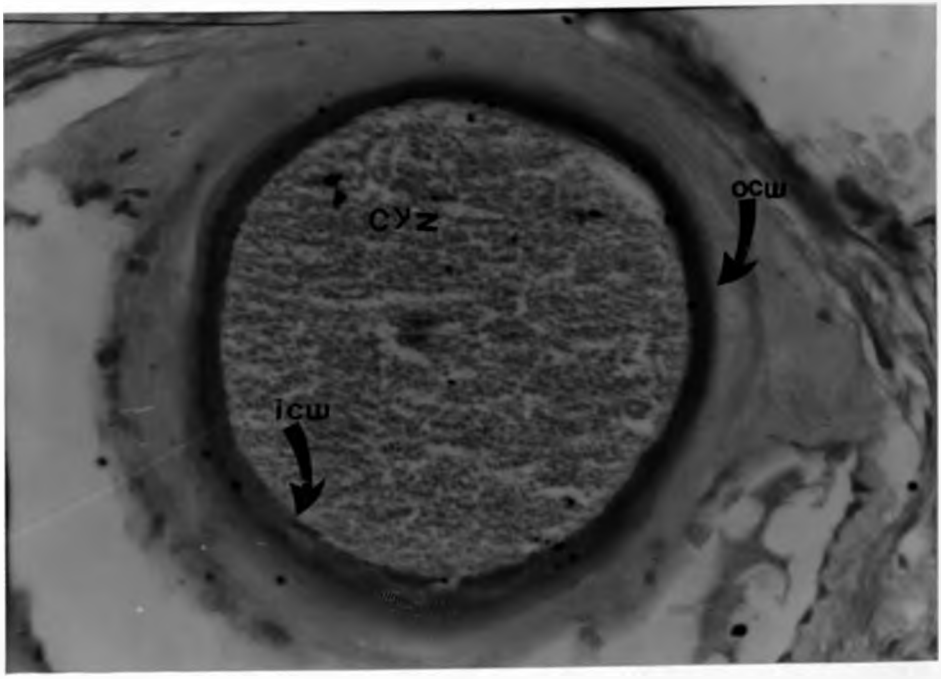


Figure 9

[9] A micrograph showing a cyst with the gram negative highly granular maroon cystozoites (cyz). The thin inner cyst wall (icw) stains similar to cystozoites while the thick outer cyst wall (ocw) stains maroon then purplish as it merges with the host tissue. (Gram x400).

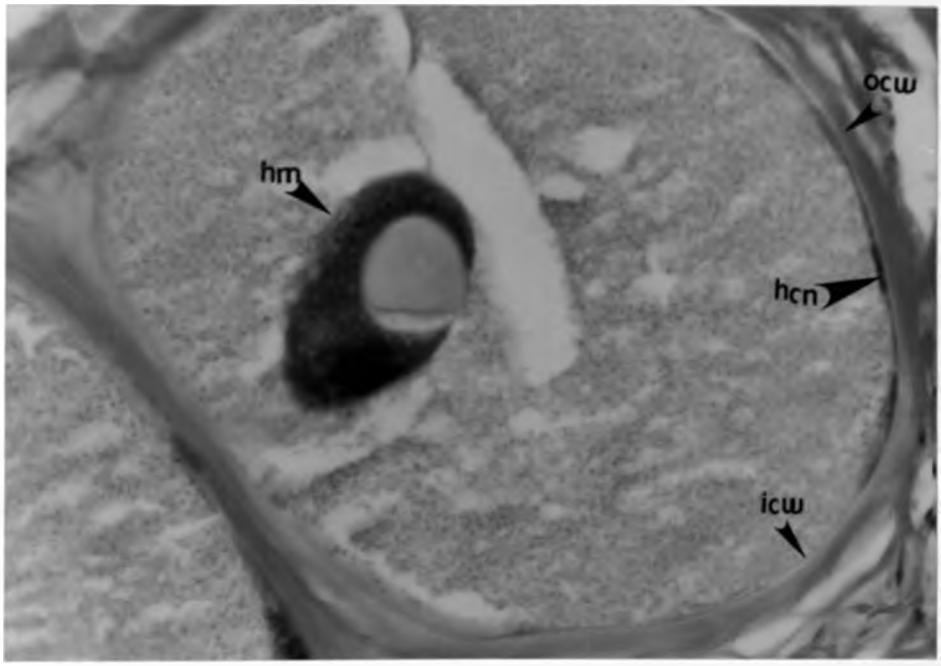


Figure 10

[10] A micrograph showing sections of cysts with the highly granular yellow to brown cystozoites. The thin inner cyst wall (icw) which stains like the cystozoites is very clear. The outer cyst wall (ocw) stains light to intense red as it merges with host tissues. The homogenous mass (hm) has a deep brown periphery and a yellow centre. The flattened dark-brown host cell nucleus (hcn) can also be seen (Van-Gieson x400).

DNA/RNA pyronin stained cystozoites light pink or intense red. The light pink appeared in almost the whole cyst except for "islands" of intense red stain that were in most cysts in the region bordering the deep and light staining zones described above for H & E, Giemsa and also seen with Periodic Acid Schiff (PAS) and Toluidine blue. The cystozoites appeared more granular in the "islands" of intense red than in the light pink zones. The inner cyst wall stained deep pink while the outer one stained very light pink and merged with host tissue, thus making it difficult to determine the exact outer cyst wall limit.

The host cell nuclei stained yellow to pink in young cysts then purple as the cyst matures. Septae formation was clear but the homogenous mass was not easily identifiable (Figure 11).

Periodic Acid Schiff (PAS) stained cystozoites intense red (Figure 12). The intensity was deeper in the periphery than at the centre. The cystozoites appear very granular. In young cysts (cysts with round host cell nucleus) the cystozoites are uniformly red but in old cysts (cysts with flattened host cell nucleus) those in the centre are lighter than the ones on the periphery. The inner cyst wall stained homogenously light red in young cysts but stained intense red in mature cysts. The outer cyst wall stained red that faded outwardly but the outer limits remain very clear.

The host cell nuclei stained dark-brown to black and the nucleolus was very clear. Septae formation and the homogenous mass were not easy to observe.

Toluidine blue (Figure 13) stained cystozoites blue-black to brown with the centre of the cyst being lighter than the periphery. In young cysts the cystozoites were reddish-brown while in old degenerating cyst they were blue-black. The necklace bead appearance of the cystozoites was very clear.

The inner cyst wall stained blue-black while the outer one stained intense red that faded outwards. Host cell nuclei stain red. The homogenous material stained brownish in the middle and green-blue in the periphery (Figure 13).

Feulgen stained cystozoites green (Figure 14) but in the very young cysts they stained greenish red. The stain was more intense on the periphery than in the centre.

The inner cyst wall stained green or greenish red in very young cysts. The outer wall stained greenish-yellow. The host cell nuclei stained maroon to purple in colour. The homogenous mass found during septae formation stains homogenously green (Figure 14).

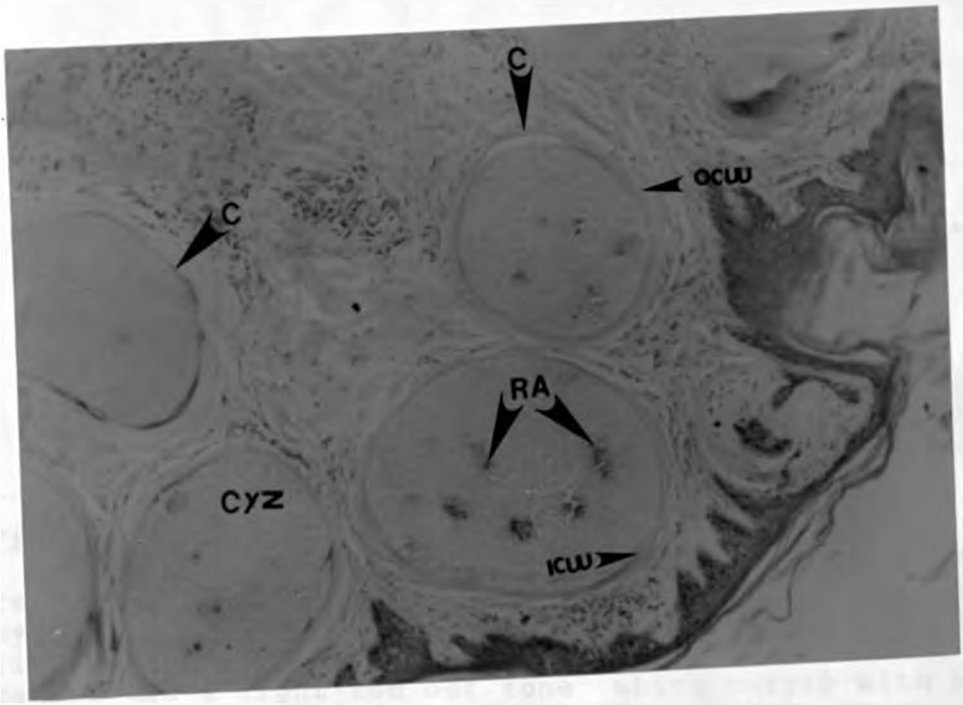


Figure 11

" A micrograph showing a section of a teat with several cysts (c) with relatively homogenous cystozoites (cyz) except for the "islands" of red areas (RA). The inner cyst wall (icw) stains red and the outer cyst wall (ocw) light pink as it merges with host tissues (DNA/RNA Pyronin x 200).

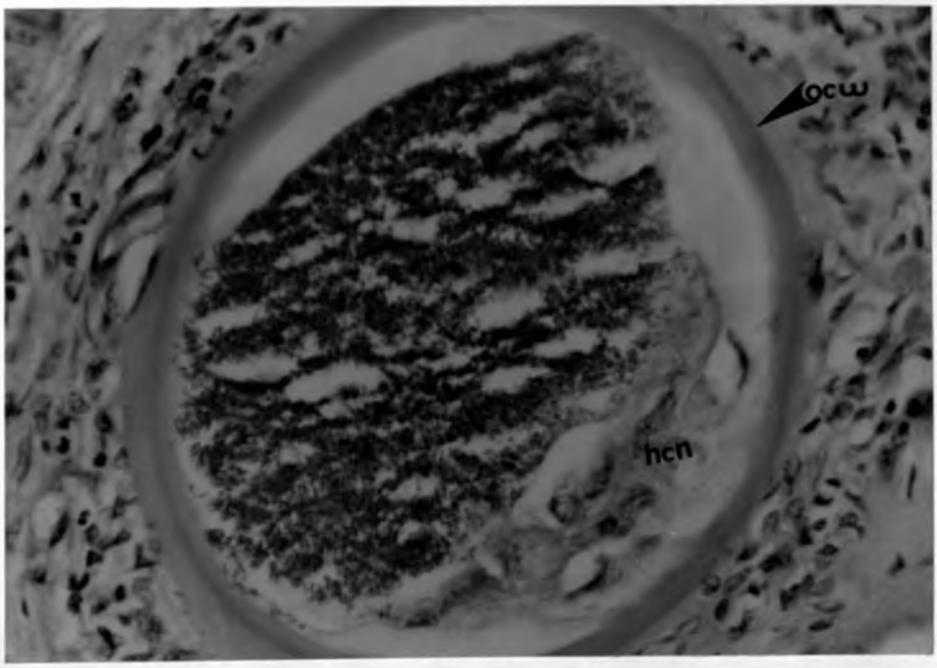
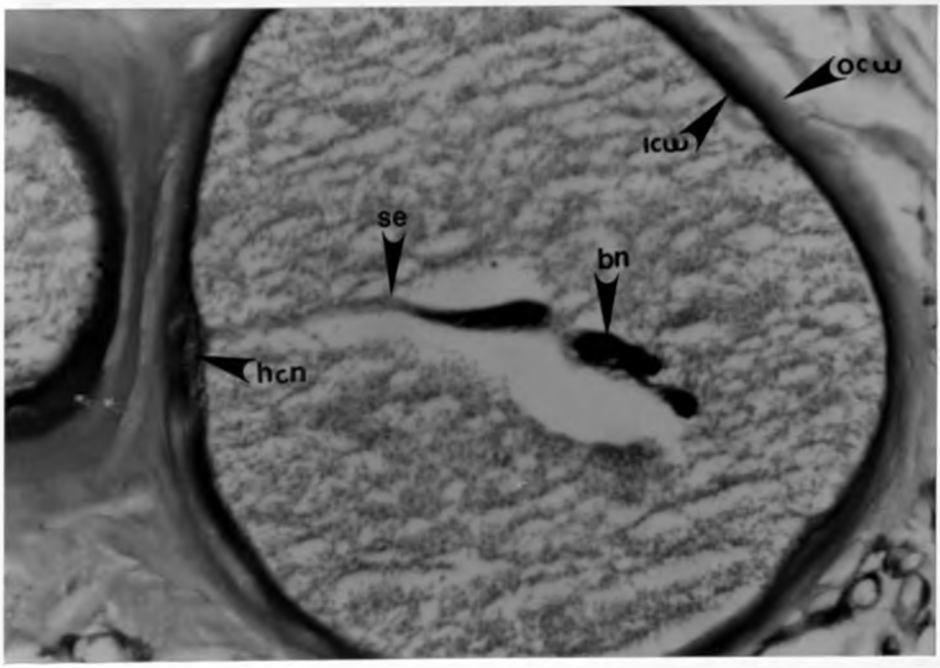


Figure 12

[12] A micrograph showing a cyst with the intense red staining highly granular cystozoites. The inner cyst wall is not clear but the outer cyst wall (ocw) is light red on the inner side followed by an intense red centre and a light red out zone which merges with host tissues. The hyperplastic multinucleate, host cell nuclei (hcn) which stain dark brown are clearly visible (Periodic Acid Schiff x400)



[13] Figure 13

A micrograph showing sections of cysts with highly granular bluish black to brown cystozoites. The inner cyst wall (icw) stains blue-black while the outer cyst wall (ocw) stains intense red that fades outwards as it merges with host tissues. The host cell nuclei (hcn) are intense red. Septae (se) formation with blackish nuclei (bn) of unclear origin are also visible (Toluidine blue x400).

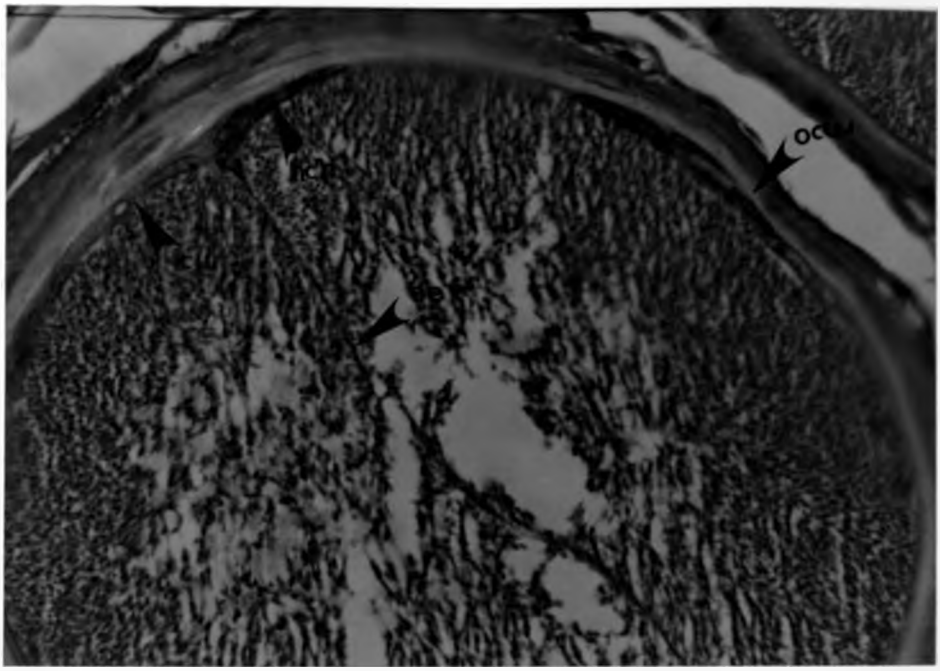


Figure 14

[14] A micrograph showing a section of a cyst with the highly granular greenish cystozoites. The inner cyst wall (icw) stains green while the outer cyst wall (ocw) stains greenish yellow and its outer limit is very clear. The hyperplastic multinucleate flattened host cell nuclei (hcn), stains maroon to purple. One of the host cell nuclei is attached to the septae (se) line. (Fulgen x400).

4.3.5 Morphometry of cysts and cystozoites

The mean diameter of the ten largest cysts per tissue and their cyst walls are given in Table 13. The lowest mean diameter was 318 µm and the highest was 618µm with an overall average of 461.4µm. The lowest mean cyst wall thickness was 10µm and the highest was 96µm with an overall average of 31.1µM.

The largest cysts were observed in the eyelids, conjuctiva and the teats while the smallest were found in the epididymis. The sizes of the cysts were most uniform in the blood vessels. Within the other tissues large differences in sizes were observed.

The freed cystozoites ranged in size from 6µm to 10.3µm in length with a mean of 8.5µm and 1.9 to 3.1µm in thickness with a mean of 2.4 µm (Table 14). Long cystozoites were generally thinner and the short ones thicker.

Table 13: The Mean Diameter Measurements of 10 Largest Cysts and Their Walls in different Ti

TISSUE EXAMINED	MEAN CYSTS DIAMETER OF 10 LARGEST CYSTS(um)	MEAN THICKNESS CYST WALLS OF THE 10 LARGEST CYSTS(um)
1. MUZZLE	578	28
2. LIPS	510	19
3. FACE	462	26
4. BRISKET	433	57
5. CARPAL	501	48
6. EARS	462	29
7. THORAX	433	49
8. HOCK	501	46
9. TRANSVERSE FACIAL VEIN	404	19
10. RADIAL VEIN	404	19
11. SAPHENOUS VEIN	404	19
12. DIGITAL ARTERIES	404	19
13. BACKLINE	597	10
14. NECK	385	19
15. SHOULDER	424	29
16. TAIL BASE	481	39
17. MAMMARY GLAND	491	29
18. TEAT	618	10
19. PAMPINIFORM PLEXUS	366	58
20. TUNICA VAGINALIS	558	19
21. TUNICA ALBUGENIA	501	77
22. EPIDIDYMIS	318	29
23. SCROTUM	501	28
24. CERVIX	385	48
25. VAGINA	462	29
26. VULVA	481	19
27. GUMS	347	29
28. SOFT PALATE	433	19
29. PHARYNX	462	19
30. TONGUE	366	48
31. ANUS	337	96
32. NOSTRIL	385	28
33. NASAL SEPTUM	412	29
34. TURBINATES	501	28
35. EPIGLOTTIS	466	36
36. LARYNX	408	46
37. TRACHEA	392	28
38. INTERCOSTAL MUSCLES	369	32
39. CUTENOUS TRUNKII.	418	26
40. LONGISMUSS DORSI	515	28
41. LATISMUS DORSI	515	28
42. PELTORAL	411	19
43. BICEPS	376	28
44. TRICEPS	412	32
45. EYELID-(UPPER)	618	10
46. CONJUCTIVA	618	14
47. EYELID(LOWER)	514	26
48. IRIS	422	29
49. PRE-SCAPULAR LYMPH NODE	501	28
50. INGUINAL LYMPH NODE	501	28
MEAN	461.4	MEAN 31.1

Table 14: Measurements of 140 Cystozoites in Microns

Length x Breath	Length x Breath	Length x Breath	Length x Breath	Length x Breath
(1) 6.7 x 2.2	(33) 8.4 x 2.2	(65) 7.7 X 1.9	(97) 7.4 x 2.8	(129) 9.4 x 1.9
(2) 6.5 x 2.2	(34) 7.4 x 2.4	(66) 8.4 x 2.2	(98) 8.9 x 1.9	(130) 8.4 x 2.6
(3) 8.4 x 2.4	(35) 7.4 x 2.4	(67) 8.2 x 2.4	(99) 9.1 x 2.2	(131) 7.2 x 2.8
(4) 8.2 x 1.9	(36) 7.4 x 2.4	(68) 7.2 x 2.4	(100) 9.1 x 2.4	(132) 7.9 x 2.4
(5) 7.4 x 2.4	(37) 8.4 X 2.4	(69) 7.9 x 2.4	(101) 7.0 x 3.1	(133) 9.1 x 2.2
(6) 6.0 X 2.8	(38) 7.2 X 2.2	(70) 6.5 x 2.4	(102) 7.9 x 2.6	(134) 9.4 x 2.4
(7) 7.2 X 1.9	(39) 9.8 X 2.2	(71) 7.4 x 2.4	(103) 7.7 x 2.4	(135) 9.1 x 2.6
(8) 7.4 X 2.2	(40) 10.1 X 2.4	(72) 8.6 x 2.4	(104) 7.7 x 2.2	(136) 8.4 x 2.4
(9) 7.9 x 2.4	(41) 8.6 x 2.4	(73) 7.7 x 1.9	(105) 7.7 x 2.4	(137) 7.4 x 2.6
(10) 7.2 x 2.4	(42) 9.1 x 2.6	(74) 8.2 x 2.2	(106) 8.6 x 2.6	(138) 7.2 x 2.8
(11) 8.2 x 2.4	(43) 7.7 x 2.8	(75) 9.6 x 2.8	(107) 7.9 x 2.4	(139) 7.0 x 3.1
(12) 8.2 x 2.4	(44) 8.2 x 2.2	(76) 9.4 x 2.6	(108) 7.4 x 2.4	(140) 7.4 x 2.4
(13) 8.4 x 2.4	(45) 9.6 x 2.2	(77) 7.2 x 2.4	(109) 7.0 x 3.1	
(14) 6.5 x 3.1	(46) 9.6 x 1.9	(78) 8.6 x 1.9	(110) 7.2 x 2.4	
(15) 7.2 x 2.2	(47) 7.2 x 2.6	(79) 9.8 x 2.2	(111) 7.7 x 2.6	Mean 8.5 x 2.4
(16) 7.4 x 1.9	(48) 6.7 x 2.4	(80) 7.7 x 2.4	(112) 7.2 x 2.4	
(17) 9.8 x 2.6	(49) 10.1 x 3.1	(81) 8.2 x 2.8	(113) 7.0 x 2.6	
(18) 9.8 x 2.4	(50) 9.6 x 2.2	(82) 9.6 x 3.1	(114) 7.4 x 2.6	
(19) 9.8 x 2.2	(51) 7.9 x 2.2	(83) 7.4 x 2.4	(115) 7.7 x 2.4	
(20) 10.3 x 2.6	(52) 7.4 x 2.4	(84) 8.6 x 2.4	(116) 9.8 x 1.9	
(21) 8.4 x 3.1	(53) 7.9 x 2.4	(85) 7.2 x 1.9	(117) 10.3 x 2.4	
(22) 7.2 x 2.4	(54) 7.4 x 2.6	(86) 7.4 x 2.2	(118) 9.1 x 2.2	
(23) 7.4 x 2.2	(55) 8.9 x 2.8	(87) 6.7 x 3.1	(119) 8.9 x 2.4	
(24) 7.2 x 2.2	(56) 7.4 x 2.4	(88) 8.9 x 2.6	(120) 7.7 x 2.6	
(25) 8.4 x 2.4	(57) 9.1 x 1.9	(89) 8.4 x 1.9	(121) 8.6 x 2.4	
(26) 8.4 x 2.4	(58) 8.6 x 2.4	(90) 7.4 x 2.4	(122) 7.9 x 2.6	
(27) 7.4 x 2.4	(59) 9.8 x 3.1	(91) 7.7 x 2.6	(123) 8.4 x 2.4	
(28) 7.4 x 2.2	(60) 7.7 x 2.6	(92) 7.2 x 2.4	(124) 8.6 x 2.8	
(29) 9.8 x 2.2	(61) 7.4 x 2.8	(93) 6.7 x 2.8	(125) 7.4 x 2.4	
(30) 7.4 x 2.4	(62) 9.4 x 2.4	(94) 7.4 x 2.6	(126) 7.2 x 2.4	
(31) 7.2 x 2.4	(63) 8.9 x 1.9	(95) 8.6 x 2.4	(127) 7.9 x 2.4	
(32) 8.2 X 2.4	(64) 7.0 X 2.2	(96) 7.2 x 2.6	(128) 9.1 x 2.6	

4.3.6 Bacteriology

The lung tissues submitted for bacteriology yielded Pasteurella haemolytica in eight of the samples (61.4 percent) while the other five did not yield anything, even after prolonged incubation.

4.4 Experimental infections of laboratory animals and domestic ruminants with caprine besnoitia

4.4.1 Clinical observations

None of the rabbits, guinea pigs, rats, hamsters, mice or bulls showed any clinical sign of the disease. Their demeanour, appetite, temperature, body condition remained normal through out the observation period of six months.

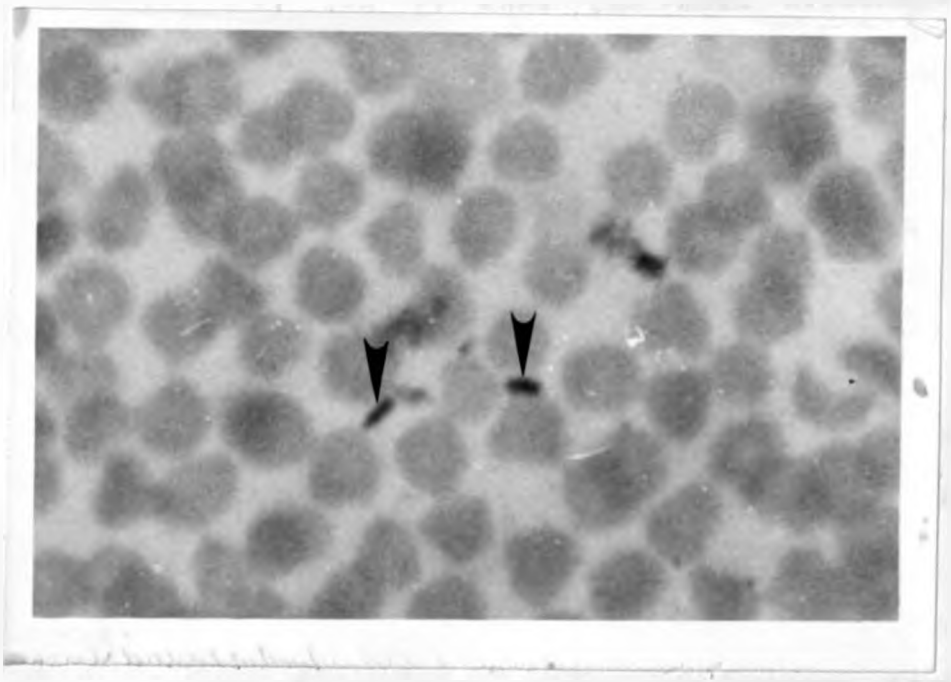
No cysts were observed in the conjuctiva or external nares of rabbits, guinea pigs, rats, hamsters, mice or bulls. The blood, buffy coats and peritoneal smears did not contain any bradyzoites. No Besnoitia cysts were observed from all the tissue sections made from these animals.

Clinical besnoitiosis occurred in all the 12 goats inoculated but not in the controls.

Three of the infected goats (25 per cent) developed a mild fever (40 - 40.5 °C), anorexia, a dull demeanour and a slight subcutenous oedema on the head and limbs 14 days after inoculation. These signs lasted for 3 days, after which the animals' temperatures returned to normal

(39.2°C), their demeanour brightened, their appetite returned and the oedema disappeared.

During the period of fever and for 3 days thereafter, bradyzoites were observed in the blood of these three goats (Figure 15). Subinoculation of 10 ml blood from these three goats on day 16 into three healthy goats produced clinical besnoitiosis with cysts appearing on the conjuctivae on day 25 after subinoculation. However subinoculation of the same amount of blood from the non-feverish infected animals to the healthy ones did not produce disease.



[15] Figure 15

A micrograph showing banana shaped endozoites (arrows) free in blood. (Giemsa x1000)

All the goats inoculated with cystozoites developed Besnoitia cysts in their conjuctiva between 25 and 40 days post-inoculation. The cysts were at first transparent, miniature and dew-like in appearance but, as they matured, they appeared translucent, like unrefined sugar or white sea-sand granules, by day 70 after inoculation.

Two of the goats that had fever and were not killed cleared the cysts from their eyes by the sixth month. Histopathology on skin biopsies did not reveal cysts in their tissues. Attempt to re-infect them 9 months later with large doses of cystozoites (approximately 10×10^9 cystozoites) was not successful.

4.4.2 Haematology

Tables 16 and 17 show the mean alternate day haematological values for the test and control goats respectively. The analysis of variance between the two showed that there was no significant difference between the test and the control animals.

4.4.3 Buffy Coat

Extensive examination of all the buffy coat smears from rabbits, ruminants and rodents did not reveal any bradyzoites.

4.4.4 Peritoneal smears

Examination of all peritoneal smears from the rodents did not reveal any besnoitia intracellularly or free in the peritoneal fluid.

Table 16: Mean Haematological Values for 12 Test Goats

Day	RBC	WBC	PCV	HB	TP	N	L
0	15.6	14.9	28.4	11.0	7.2	33	64
2	14.7	15.0	29.0	10.1	7.2	36	62
4	15.1	14.9	29.2	10.1	7.4	36	63
6	15.4	13.7	28.6	10.2	7.4	33	63
8	15.6	14.1	28.5	9.9	7.3	36	62
10	15.6	14.1	29.0	9.6	7.4	37	61
12	15.9	14.2	28.4	9.9	7.2	37	62
14	15.8	14.4	28.6	9.5	7.5	31	66
16	15.5	12.7	28.0	10.4	7.4	34	64
18	16.4	13.7	28.9	9.6	7.4	33	63
20	15.0	14.9	29.1	9.5	7.5	31	68
22	15.3	14.3	28.4	9.5	7.1	34	64

Note:

RBC = Red Blood Cell (P-Value = 0.86)
WBC = White Blood Cell (P-Value = 0.96)
PCV = Packed Cell Volume (P-Value = 0.92)
HB = Haemoglobin (P-Value = 0.95)
TP = Total Protein (P-Value = 0.99)
N = Neutrophils (P-Value = 0.94)
L = Lymphocytes (P-Value = 0.91)

Day 0 - Means of 5 alternate days before start of experiment .

P Value < 0.05 the difference is significant
P Value > 0.05 the difference is not significant

Table 17: Mean Haematological Values of 4 control Goats

Day	RBC	WBC	PCV	HB	T	N	L
0	14.4	15.8	28.8	11.1	7.3	32	68
2	15.1	14.6	29.0	10.9	7.2	37	60
4	15.5	13.7	29.0	10.6	7.3	30	67
6	15.6	14.5	29.0	9.9	7.2	33	66
8	15.5	14.8	29.2	10.2	7.3	36	62
10	15.0	14.7	30.0	10.0	7.1	33	64
11	15.6	4.8	27.0	9.6	7.5	31	66
12	15.6	14.7	30.0	9.7	7.2	34	62
14	15.5	14.3	27.5	9.9	7.4	36	61
16	15.2	15.4	29.0	9.7	7.3	33	64
18	15.1	14.3	30.0	9.6	7.1	34	61
20	15.7	14.6	29.0	9.7	7.4	32	66
22	15.1	14.8	29.0	9.6	7.4	31	66

RBC = Red Blood Cell (P-Value = 0.86)
WBC = White Blood Cell (P-Value = 0.96)
PCV = Packed Cell Volume (P-Value = 0.92)
HB = Haemoglobin (P-Value = 0.95)
TP = Total Protein (P-Value = 0.99)
N = Neutrophils (P-Value = 0.94
L = Lymphocytes (P-Value = 0.91)

Day 0 – Means of 5 alternate days before start of experiment i.e. baseline value

P Value < 0.05 the difference is significant
P Value > 0.05 the difference is not significant

4.4.5 Gross pathology

All the animals killed had good body condition and had no detectable abnormalities. No besnoitia cysts were observed in rabbits, rodents and bulls.

The goats, even though they had good body condition, had masses of besnoitia cysts in the dermis fascia, tunica albuginea, muzzle, pampinform plexus and teats. Relatively few cysts were found in the heart, lungs, spinal cord, and gastrointestinal tract. No cysts were seen in the kidney or liver.

4.4.6 Histopathology

Histopathology confirmed the gross observations. No besnoitia cysts were observed in tissue sections from rabbits, rodents and bulls.

In the goats cysts were observed and their density was as seen on gross examination. Cysts at different stages of development were observed. Some elicited no inflammatory reaction while others elicited severe reaction with heavy presence of polymorphonuclear leukocytes around them (Figure 16).

Figure 16
[16] A micrograph showing the subcutis of a doe that was experimentally infected with caprine besnoitia. There are three cysts (c) at different stages of development. One of the cysts has septae (se) forming. The cysts have triggered a severe inflammatory reaction with massive infiltration of inflammatory cells (ifc). (H&E x 200 - 70 days post inoculation).

4.5 Comparative experimental infectivity studies of *Besnoitia besnoiti* and caprine besnoitia

4.5.1 *Besnoitia besnoiti*

4.5.1.1 *Clinical observations*

The two rabbits inoculated with *B. besnoiti* developed fever of 40.5°C ten days post-inoculation, and this rose to 42°C by the thirteenth day. The fever remained high (>40.5°C) for 8 days. During the period of fever the animals developed mucopurulent ocular discharge, anorexia and dullness, and the male had severe scrotal oedema. The testes were painful to touch. Blood and buffy coat smears did not reveal bradyzoites. The oedema then disappeared, the appetite improved moderately but the mucopurulent ocular discharge persisted.

Two months later, the two rabbits started developing alopecia on the ears and this extended to the neck. The alopecia was followed by cracking of the skin, severe necrosis and ulceration by the fourth month. The mucopurulent ocular discharge increased during the same period. The rabbits became paralysed, could not move, eat and had to be euthanised (Figure 17).

All the goats developed clinical besnoitiosis. They developed fever of 41°C, 11 days post-inoculation, anorexia, subcutenous oedema and dullness. Blood smears did not reveal bradyzoites. Besnoitia cysts appeared on the conjuctiva on the 37th day post-inoculation.

The bulls developed mucopurulent ocular discharge one day post-inoculation and this persisted for three weeks. The two bulls developed fever of 39.7°C, nine

Figure 17

A photograph showing a rabbit suffering from chronic besnoitiosis. The legs are paralysed and the animal is on sternal recumbency. The ear and the back of the neck are alopecic and necrotised. Fissures and ocular discharge are also visible (4 months post-inoculation)

Figure 18[18] A photograph of the eye of a bull experimentally infected with B. besnoiti showing very small white spots that represent besnoitia cysts (arrow) on the scleroconjuctiva (3 months post inoculation).

days post-inoculation. The fever persisted for 8 days thereafter before returning to normal. Slight subcutenous oedema was noticed in the two bulls during the period of fever. No bradyzoites were observed in blood and buffy coat smears. Besnoitia cysts became visible in the scleral conjuctiva by day 22 (Figure 18). The animals started losing their body condition and on the second month post-inoculation, while skin folds were observed by the third month.

4.5.1.2 *Gross pathology*

In rabbits, in addition to the alopecia, ulcerations and necrosis on the ears and neck, poor body condition was observed in the two infected rabbits. The ears were full of crusts that blocked the whole auditory canal. Deep fissures were found in the ears and on the neck. Areas bordering the necrosed areas had erythema.

The skin in the ulcerated areas was thick. Flaying of the skin showed that the lesion did not penetrate into the muscles and only involved the skin and subcutis.

The goats had good body condition. Besnoitia cysts were few in the subcutis and fascia. No other lesions were found in these animals.

In bulls slight loss of body condition, folding of the skin and presence of cysts on the scleroconjuctiva, subcutis, and fascia was observed.

4.5.1.3 *Histopathology*

In rabbits examination of the skin and subcutis revealed necrosis, ulcerations and deep fissures (Figure 19). The normal skin structure was completely destroyed in the affected areas. However in the unaffected areas, the skin looked normal. Other areas were free of lesions.

Figure 19

A micrograph showing the skin of a rabbit suffering from chronic B. besnoiti infection. Necrotic tissue (nt) and deep fissures (f) and complete loss of normal skin tissue is seen in this section (H&E x 40 - 4 months post-inoculation).

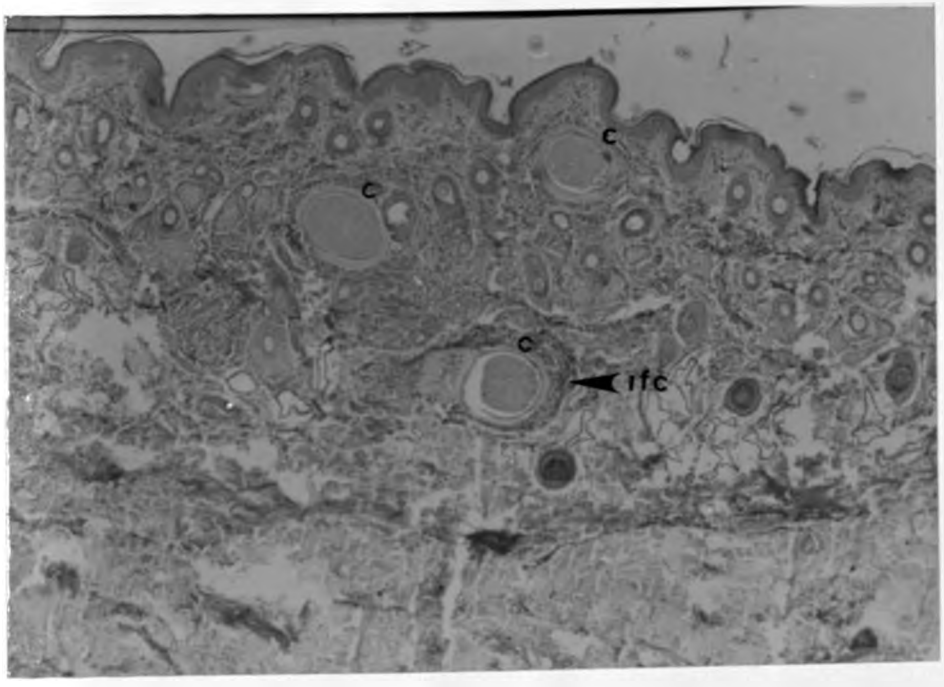


Figure 20
[20] A micrograph showing a section of a goat skin with three B. besnoiti cysts (c). There is heavy inflammatory cell (ifc) infiltration around the cysts (H & E x 100 - 4 months post-inoculation).

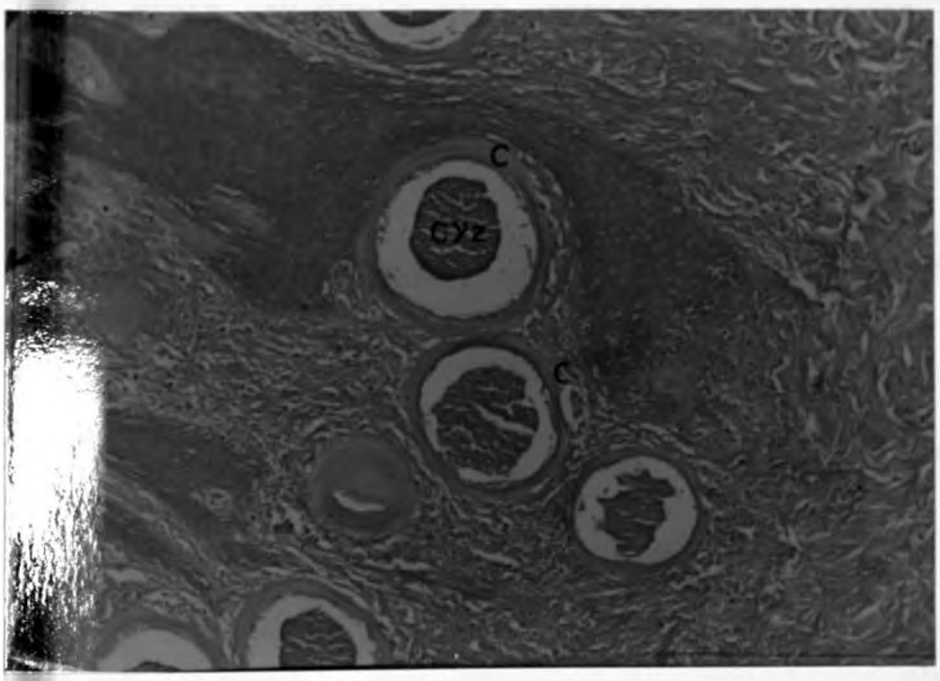


Figure 21
[21] A micrograph of a skin section showing several cysts (c) with cystozoites from an experimentally infected bull 6 months post inoculation. The cysts have not provoked any inflammatory reaction and the cystozoites (cyz) stain uniformly red (PAS x200).

In goats few cysts were observed in the areas that grossly showed cysts. The cysts had thick walls and elicited inflammatory reaction. This was characterised by presence of inflammatory cells around the cysts. No other lesions were observed in the tissues of infected goats (Figure 20).

In bulls many cysts were observed in the skin and subcutis (Figure 21). The cysts were of varying sizes. Most of the cysts elicited inflammatory reactions but others did not. No other lesions were observed.

4.5.2 Caprine besnoitia

4.5.2.1 *Clinical observations*

All the rabbits remained healthy throughout the observation period.

The goats remained bright throughout the observation period. However they all developed scleroconjuctival besnoitia cysts between day 25 to 37 post inoculation.

The bulls remained healthy throughout the observation period. No cysts developed in the scleroconjuctiva of the bulls.

4.5.2.2 *Gross Pathology*

No lesions were observed in the killed rabbits.

The goats had good body condition but had besnoitia cysts distributed in the body as described in section 4.3.2. While in bulls, no lesions were observed in the tissues.

4.5.2.3 *Histopathology*

No cysts or other lesions were observed in rabbits.

In goats the lesions observed were similar to those reported in section 4.3.3.

In bulls the observations made in this experiment were similar to those made in section 4.4.6.

4.6 Infection of rabbits and cattle previously exposed to caprine besnoitia with B. besnoiti

4.6.1 Clinical Observations

Both the rabbits and bulls previously inoculated with caprine besnoitia did not develop any clinical signs. They retained their appetite and body condition. No Besnoitia cysts were observed in their eyes or on skin biopsies during histopathology.

The two rabbits and two bulls that had no previous exposure to B. besnoiti developed clinical besnoitiosis which took a similar course as that described in section 4.5.1.1.

4.6.2 Gross pathology

The gross pathology in previously unexposed rabbits and cattle was similar to that observed in section 4.5.1.2. No lesions were observed in the previously exposed animals.

4.6.3 Histopathology

Histopathology confirmed the presence of B. besnoiti cysts in cattle previously unexposed to Caprine besnoitia. In previously unexposed rabbits the lesions found were similar to those reported in section 4.5.1.3 However no lesions were found in previously exposed rabbits.

4.7 Experimental infection routes of goats with caprine besnoitia

4.7.1 Implantation

The goats remained bright throughout the observation period of six months. They had good appetite. However from the third day the prescapular lymph nodes on the sides where fascia implantation was done started swelling while that of the other side was normal. On day 24 the two animals showed a very large number of miniature dew-like cysts in their conjuctiva.

One of these animals later gave birth to a healthy kid which did not have cysts in the eyes or skin. Presence of cysts in the two does was confirmed on histopathology.

4.7.2 Needle pricking

The two goats were slightly depressed and reluctant to move during the first three days post-pricking. Their appetite was moderate. However they brightened and their appetite improved by the sixth day. Besnoitia cysts appeared in the scleral conjuctiva of the two goats on day 37 post-pricking and were confirmed on histopathology.

4.7.3 Intrauterine

Besnoitia cysts were observed in the skin of the euthanised chronically and freshly infected does. However none of the fetuses showed any cyst either grossly or on histopathology.

Two kids, one born from the chronically infected doe and the other from the freshly infected doe were normal and had no cysts in the eyes or skin tissues. An attempt to transmit the disease by inoculating the kids subcutenously using approximately 5 x 10^9 cystozoites resulted in one kid (born from the chronically sick goat) getting infected and developing conjuctival cyst but the other (born from the freshly infected doe) failing to become sick.

4.7.4 Intranasal

These two goats remained bright and had good appetite throughout the observation period. Both developed conjuctival cysts, one on day 32 and the other on day 40 post-inoculation. The cysts were few but were confirmed on histopathology. One doe kidded to a normal male.

4.7.5 Intraconjuctival

One of these goats developed a high fever of 41°C on day 16 post inoculation. The fever remained high for six days before falling back to normal (39.2°C). On the second day of fever it developed oedema on the skin, head and limbs. It aborted a normal fetus on the 4th day of fever. Bradyzoites were observed in its blood smears. The other remained bright throughout the observation period. Both goats developed conjuctival cysts on day 25 post-inoculation and were confirmed on histopathology.

4.7.6 Ocular

The two goats showed increased lacrination for about 2 hours after inoculation. This stopped and the animals remained normal throughout the observation period. None of these animals developed besnoitiosis.

4.7.7 Oral

The two goats remained bright throughout the observation period. None of these animals developed besnoitia cysts.

4.8 Pathogenesis of caprine besnoitiosis

Days 1 and 2: All the goats were bright, had good appetite and were in good body condition. The animals did not show scleral-conjuctival cysts, oedema or presence of discharges from the nose, eyes or other orifices.

Blood smears did not reveal bradyzoites. A negative control goat No. 284 was sacrificed. No gross lesions were noted on antemortem or postmortem examination. Histopathology revealed normal goat tissues.

Day 3 (No. 282 Killed): The clinical, blood and pathological features remained as they were on day 1 and 2. Histopathology revealed normal goat tissue (Figure 22)

Day 7 (No. 283 killed): The clinical, blood and gross pathological features remained as in day 3. Histopathology revealed heavy infiltration of

inflammatory cells. The venules and arterioles were congested. Very early cysts with two or three dividing host cells surrounded by a thin cyst wall were observed (Figure 23). Their mean diameters were 14μM.

Day 11 (No. 286 killed): All the animals were clinically healthy as they were on day 7. No bradyzoites were found in blood of all the animals. Gross pathology on the killed animal did not show any lesions.

Histopathology revealed enlarged more granular host cells. The cysts contained a few highly eosinophilic cystozoites and the cyst wall was better delineated. The mean cyst diameter was 24μm. Inflammatory cells were still found in large numbers in the tissue sections while arterioles and venules were still congested (Figure 24).

Day 14 (No. 289 killed): The animals remained clinically as they were on day 11. Their blood picture also remained the same without bradyzoites. The gross pathology did not reveal any lesions either. Histopathology showed cysts with a better defined cyst wall. The cyst vacuole was slightly enlarged and the host cells just as granular as they were on day 11. Cystozoites were observed free in the cyst vacuole. The mean cyst diameter was 28μm. Inflammatory cells were still found in large numbers (Figure 25).

Day 17 (No. 290 killed): Three goats, Nos.,292, 302 and 307 developed fever of 40.8°C. They became dull and had no appetite. The respiratory and heart rates increased markedly. There was mild lacrimation in the three animals. The blood of the three goats showed

banana-shaped bradyzoites.

The other animals remained bright and did not develop fever or any other clinical signs. No bradyzoites were found in their blood.

Gross pathology of the killed animal was similar to that found on day 14. Histopathology, however revealed larger cysts (mean diameter 38µm) containing cystozoites mingled with host cell nuclei without any specific pattern. There were many inflammatory cells (Figure 26).

Day 18 and 19: The three goats still had a fever of 40.8°C, were dull and anorexic. They had fast, shallow breathing and were reluctant to move. They had noticeable oedema on the face and legs. Palpation of skin revealed that the oedema was affecting the whole skin. The blood of these goats still had bradyzoites. The others remained as they were on day 17.

Day 20 (No. 292, killed): The three goats Nos. 292, 302 and 307 had a reduced fever (40.4°C). Oedema was still as it was on days 18 and 19. Hyperpnoea was still there and the appetite was poor. Goat 302 aborted on the evening of day 20. The fetus appeared normal but the placenta looked oedematous with a pale yellowish gelatinous appearance. No cysts were observed grossly or on histopathology of the skin tissues of the aborted fetus. Bradyzoites were observed in the blood of these three goats.

Gross pathology on goat 292 revealed a thickened skin with a staring coat and congestion of most organs.

Histopathology revealed larger cysts (mean diameter 41µm) with more numerous host cell nuclei and cystozoites. There were many inflammatory cells (Figure 27). The other goats remained normal and no bradyzoites were observed in their blood.

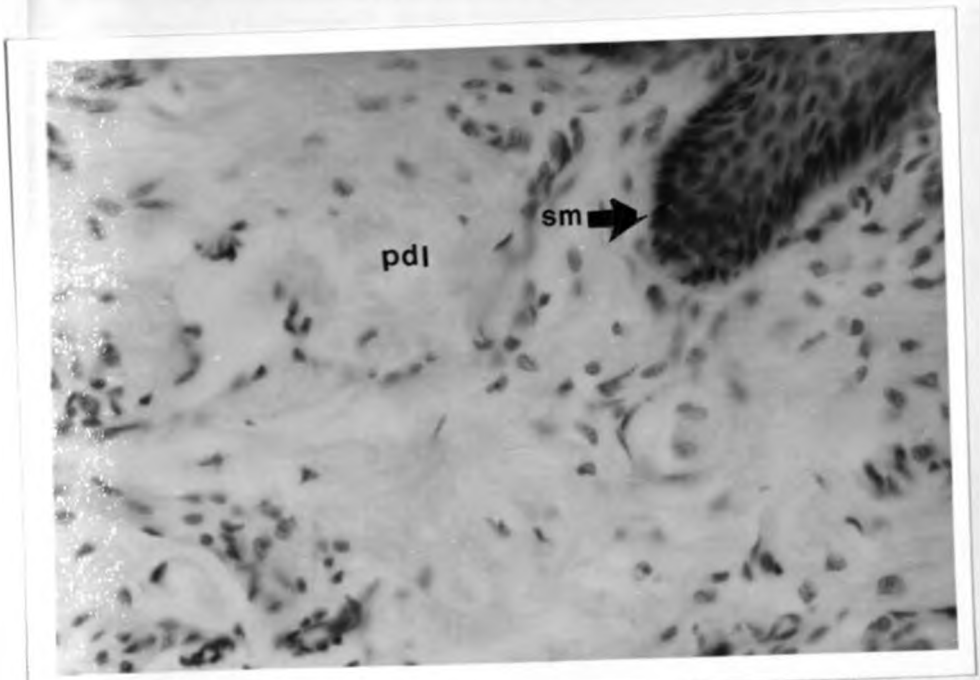


Figure 22
[22] A micrograph of the normal skin between the muzzle and the nostrils showing part of the stratum malpighii (sm) and the papillary layer of the dermis (pdl) of a goat. (H&E X 200).

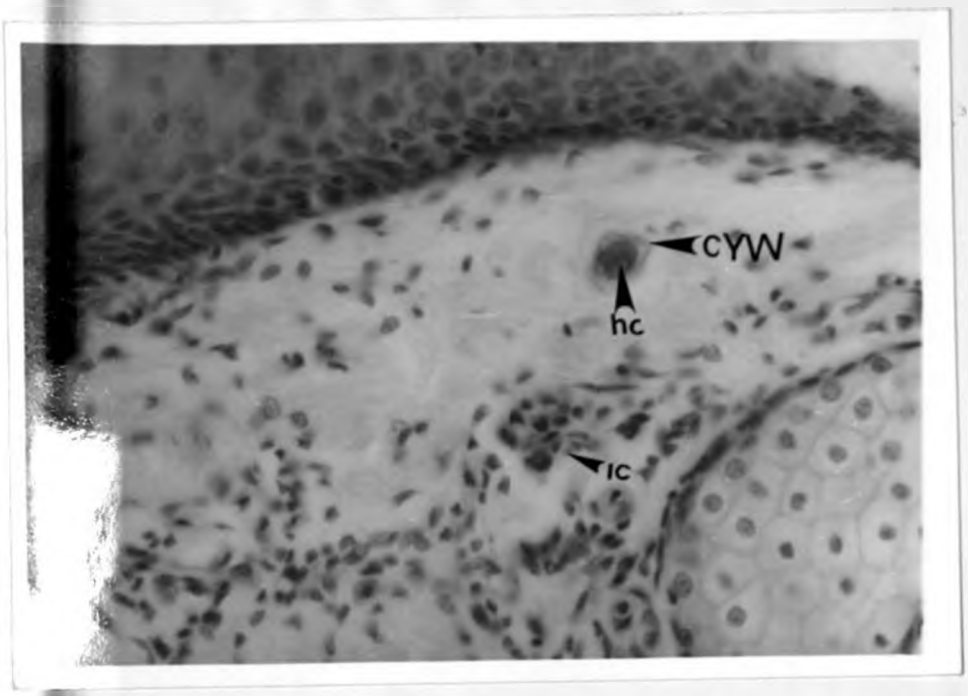


Figure 23
[23] A micrograph showing the skin between the muzzle and the nostrils of a goat. A young cyst with three host cell nuclei (hc). The cyst wall (cyw) and inflammatory cells (ic), are also visible. (H&E X 200) 7 days post inoculation.

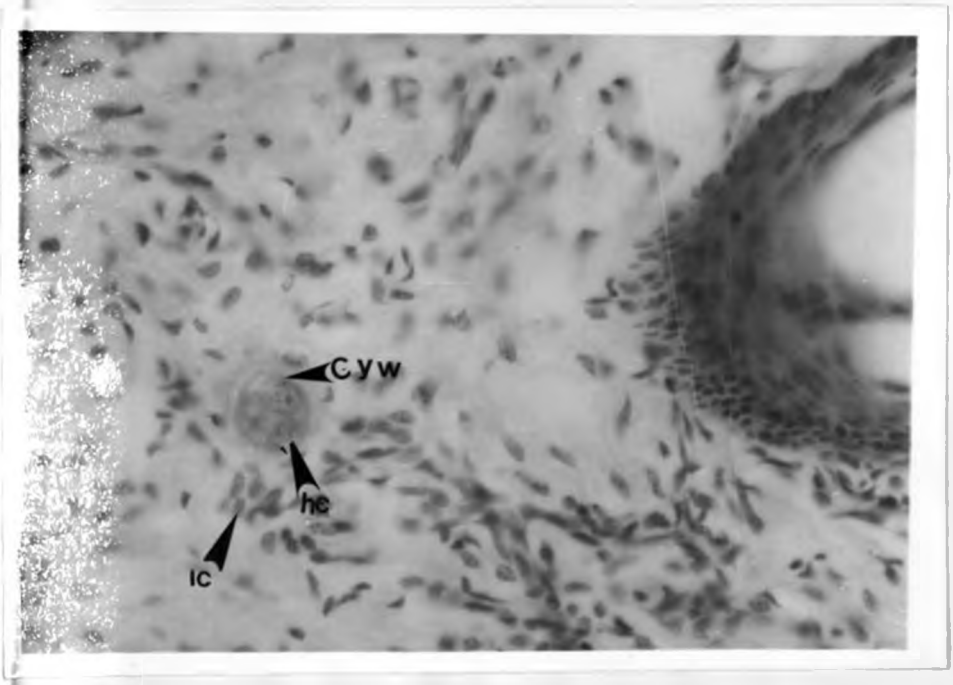


Figure 24

[24] A micrograph of skin showing a slightly larger cyst with a bigger host cell nucleus(hc). The cyst wall (cyw) is better defined than in Figure 23. Inflammatory cells (IC) are also visible. (H&E X200 11 days post-inoculation).

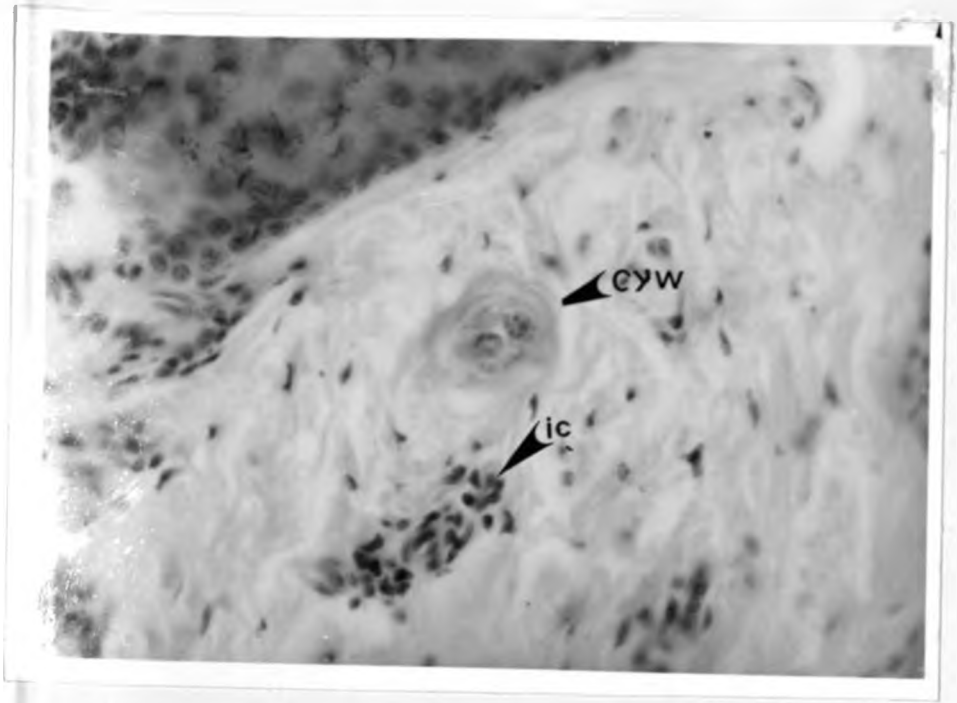


Figure 25
[25] A micrograph showing the skin of a goat with a larger cyst which has a much thicker cyst wall (cyw). Inflammatory cells (ic) are also visible. (H&E X200) - 14 days post-inoculation.

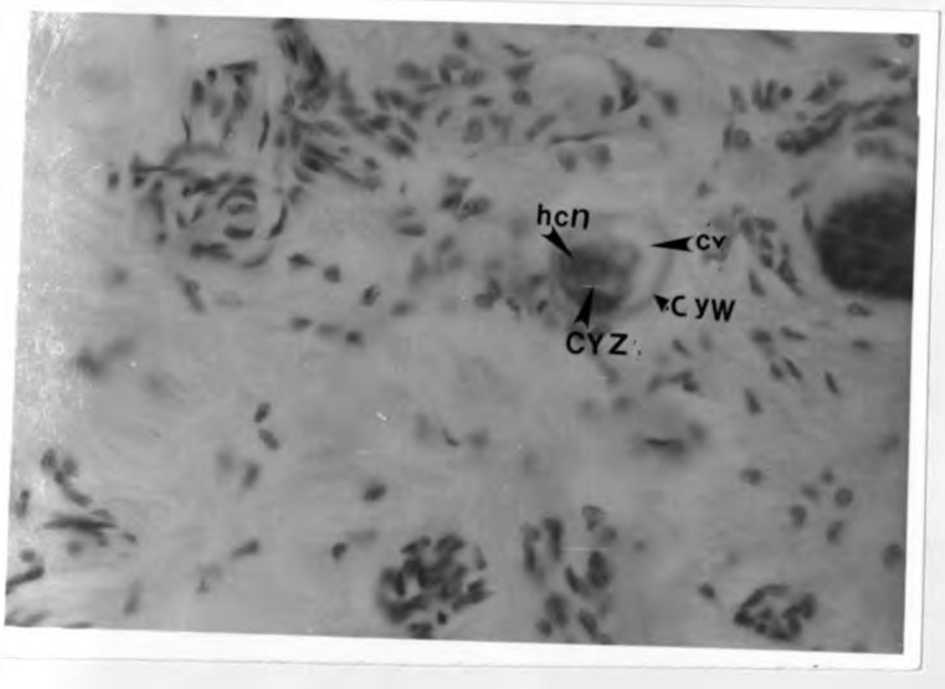


Figure 26
A micrograph showing a cyst with well defined cyst wall (cyw). The host cell nuclei (hcn) are not arranged in any pattern in the cyst vacuole (cv). (H&E X200 17 days post-inoculation).

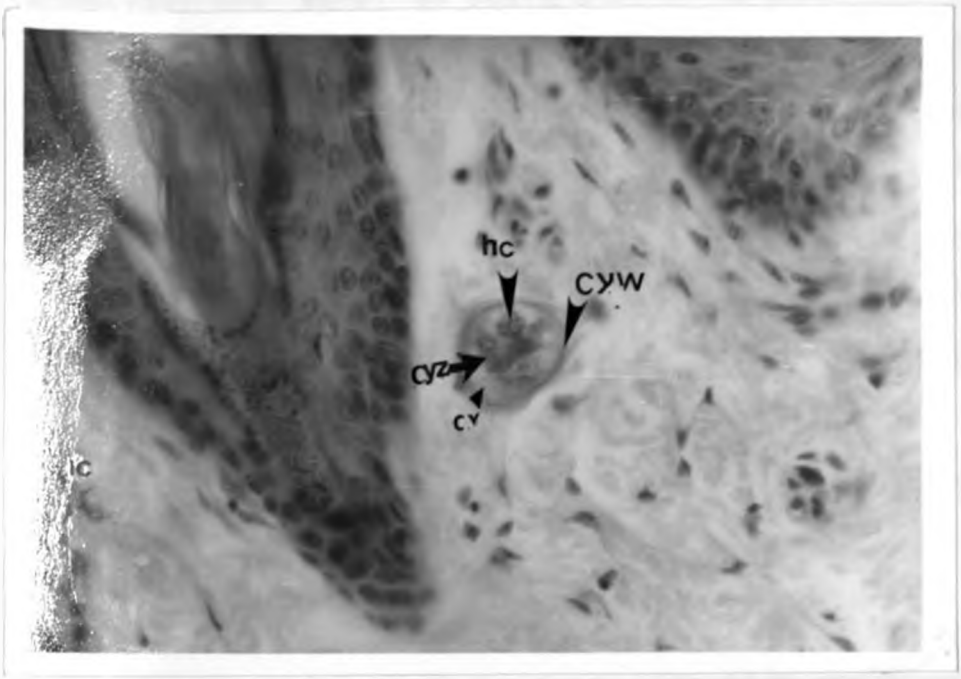


Figure 27
A micrograph showing a cyst with a well defined cyst wall (cyw), several host cell nuclei (hcn), cystozoites (cyz) and cyst vacuole (cv). Inflammatory cells (ic) are also visible (H & E x 200 - 20 days post-inoculation).

Day 21, 22: Fever in goats No. 302 and 307 was down to 40°C but the oedema persisted. Their appetite improved markedly and their demeanour brightened slightly. The hair coat was still staring. Few bradyzoites were observed in their blood. Five does Nos. 293. 298, 300, 302 and 306 developed miniature dew-like cysts in their scleral-conjuctiva. The other seven does did not reveal eye cysts.

Day 23 (No. 293 killed): The temperature of does 302 and 307 was down to 39.4°C. They were brighter, had a good appetite and oedema was markedly reduced. The coat was still staring. Scanty bradyzoites could still be observed in blood smears.

Conjuctival cysts which were miniature and dew-like in appearance appeared in does 297, 303 and 305. However these animals together with those that showed cysts the previous two days, remained normal clinically and no bradyzoites were observed in their blood.

On necropsy of No. 293, many miniature dew-like cysts were observed in the subcutis and endothelium of the superficial blood vessels of the limbs and face.

Histopathology showed cysts with well defined cyst walls, containing host cell nuclei arranged in the cyst vacuole like a beaded necklace surrounding cystozoites in the middle (Figure 28). The mean cyst diameter was 48µm. There were many giant cells and fibroblasts.

Day 26 (No. 297 killed): The temperature of does 302 and 307 was back to normal (39.2°C). They had good appetite and bright demeanour. Oedema was almost gone

but the hair coat was staring. Bradyzoites were difficult to locate in their blood but very occasionally one or two could be observed in the whole blood smear. The other goats No. 299, 301, 304 and 307 also developed miniature dew like cysts in their conjuctiva thus bringing to 100 per cent the number of animals with cysts. Bradyzoites could not be demonstrated in the blood of these animals.

On post mortem of No. 297 more conspicuous cysts were observed in the subcutis and endothelium of the superficial blood vessels of the limbs and face. Histopathology showed cysts that were much larger (mean diameter 58µm) but with a similar arrangement of the host cell nucleus and cystozoites like that of day 23. However there was a marked increase in the number of cysts whose outer wall was heavily invaded by inflammatory cells, namely neutrophils, eosinophils and monocytes (Figure 29).

Day 29 (No. 298 killed): All the animals were bright, had good appetite and oedema had fully disappeared in No. 302 and 306. The hair coats of these two goats however were still staring but not as much as they were on day 26. Conjuctival cysts were more conspicuous than on day 26. No bradyzoites could be observed from any of the goats. The post mortem of goat 298 revealed a similar picture to that observed on day 26 except that the cysts were more conspicuous.

Histopathology showed larger cysts (mean cyst diameter 67µm). In many cysts, the beaded necklace

arrangement of the host cell nuclei was broken and the nucleus pushed towards the periphery by the increased cystozoites. The inflammatory cells appeared to be migrating from the cysts and were widely distributed around the cyst (Figure 30).

Day 33 (No. 299 killed): The clinical and blood picture remained as it was on day 29 except for the cysts that were more conspicuous. The appearance of the cysts was like that of refined sugar.

Necropsy on No. 299 showed similar features like those of day 29 except for the larger cysts.

Histopathology revealed larger cysts (mean diameter 77µm). Host cell nuclei were pushed to the periphery and the vacuole almost filled with cystozoites which were arranged in stacks. Giant cells and fibroblasts and a few polymorphonuclear cells were observed (Figure 31).

Day 40 (No. 300 killed): The animals remained clinically as they were on day 33 except that the cysts were more conspicuous. No bradyzoites were observed in blood smears of all goats.

Histopathology revealed very large cysts (mean diameter 96µm) surrounded by giant cells and fibrocytes. Thrombosis was observed in some venules and arterioles. Other early developmental stages of the cysts described previously were also seen (Figure 32).

Day 46 (No. 301 killed): The clinical and blood picture of the animals remained as they were on day 40 except that the cysts were becoming translucent.

The necropsy features were similar to those of day 40 except that the old cysts were bigger (mean diameter 144µm). Other early developmental stages were also seen.

Day 56 (No. 302 killed): Clinical, blood, gross and histopathological features remained the same as in day 46, except that the cysts were much bigger (mean diameter 212µM) and less translucent. Most of the cysts were surrounded by fibroblasts and few inflammatory cells (Figure 33).

Day 66 (No. 303 killed). The clinical, blood and gross pathological features remained as they were on day 56 except that the cysts were much bigger (mean diameter 308µm). There were many mature cysts on histopathology and few of the early stages observed before day 23. Host cell nuclei were pushed to the periphery and flattened in appearance (Figure 34).

Day 76 (No. 304 killed): The clinical, blood and gross pathological features remained as they were on day 66. The cysts were translucent like unrefined sugar or white sea sand granules.

Histopathology showed many mature cysts like the ones reported earlier during study on natural Caprine besnoitiosis, with host cell nuclei pushed to the periphery and flattened by the cystozoites which filled the whole vacuole. The mean cyst size did increase markedly from day 66 (mean diameter 433µm). There were fewer cysts showing the earlier stages than those seen on day 66.

Day 86 (No. 305 killed): The clinical, blood and gross pathological features remained as they were on day 76. Histopathology revealed mature cysts whose mean diameter was 481µm. There were very few immature cysts compared to day 76. The nuclei of the host cells were even more flattened. In some cysts septae formation was observed.

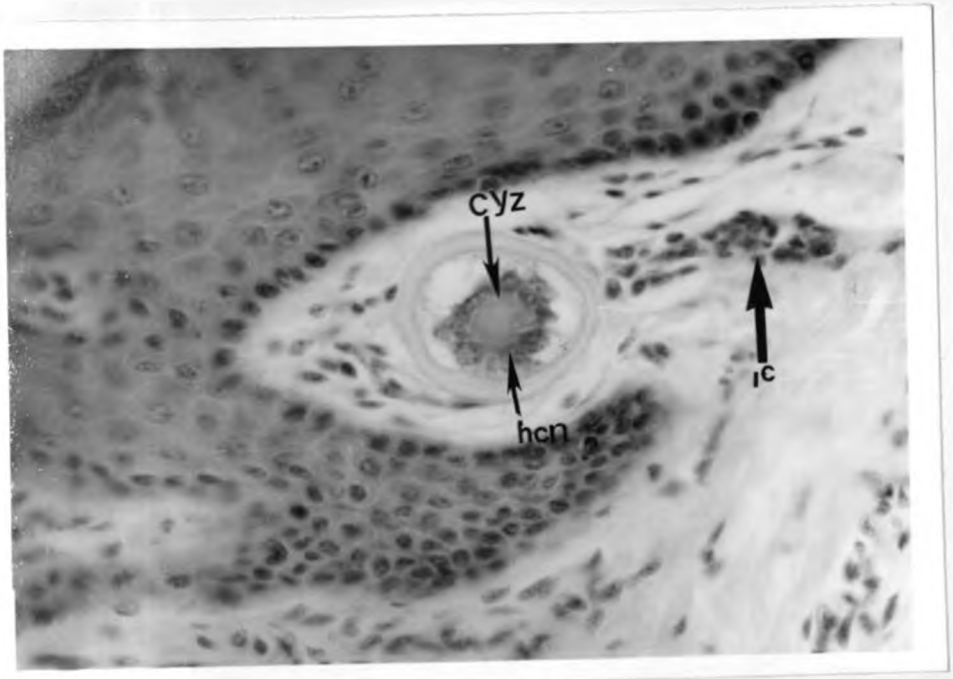
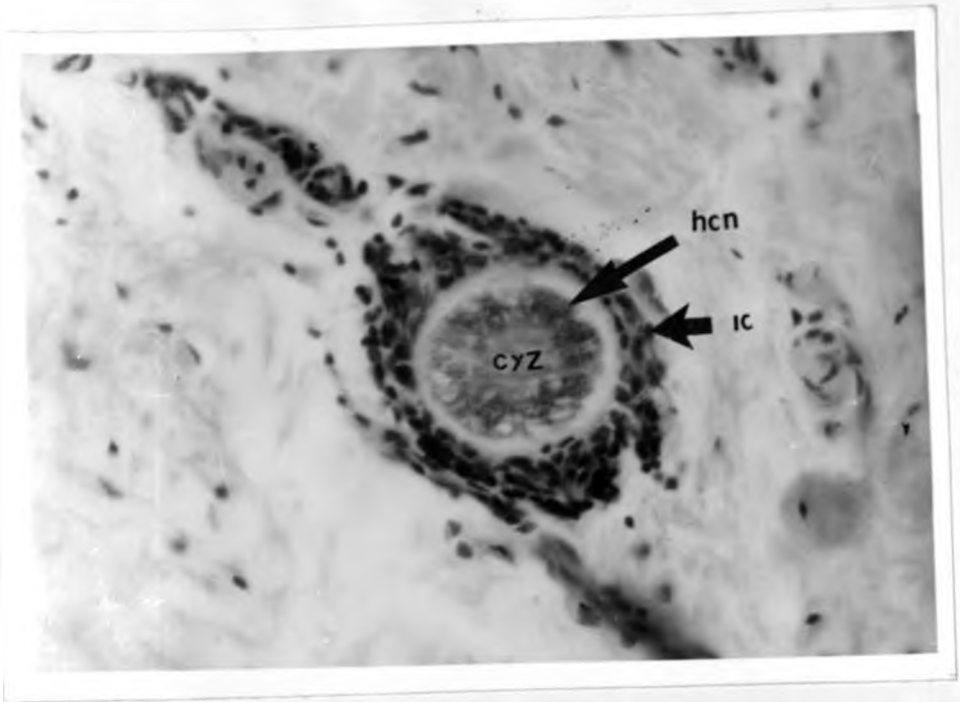


Figure 28
[28] The skin showing a well defined cyst with many host cell nuclei (hcn) arranged like beads around increased cystozoites (cyz). Inflammatory cells (ic) were still present. (H&E X200 23 days post-innoculation).



[29] Figure 29

A micrograph showing a large cyst. The host cell nuclei (hcn) are arranged like beads around the cystozoites (cyz). There is a heavy peripheral infiltration of inflammatory cells (ic). H&E x200 -26 days post-inoculation.

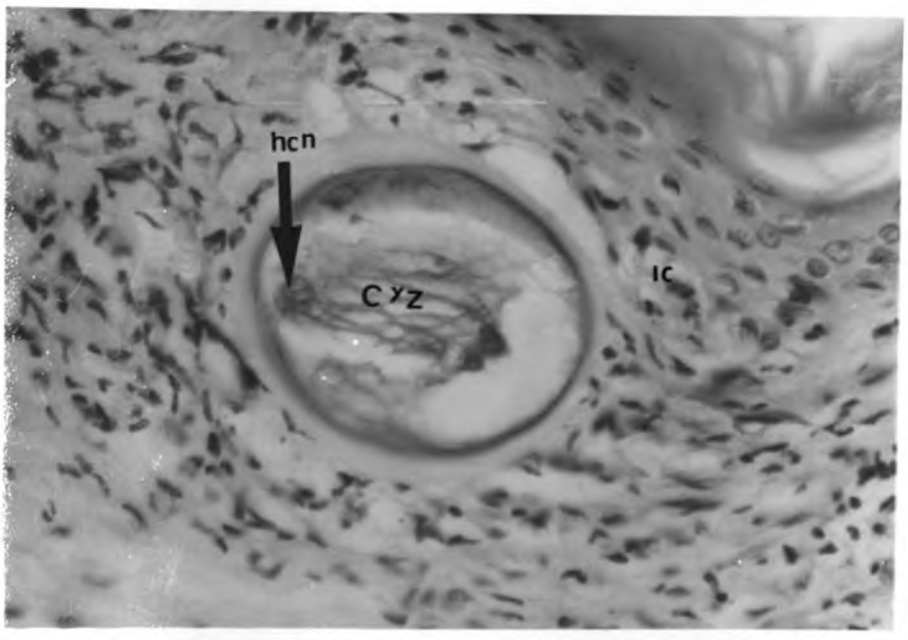


Figure 30
[30] A micrograph of a skin showing a bigger cyst with host cell nuclei (hcn) pushed towards the periphery by the increased cystozoites (cyz). The inflammatory cells (ic) are widely distributed around the cyst. (H&E X200 29 days post-innoculation).

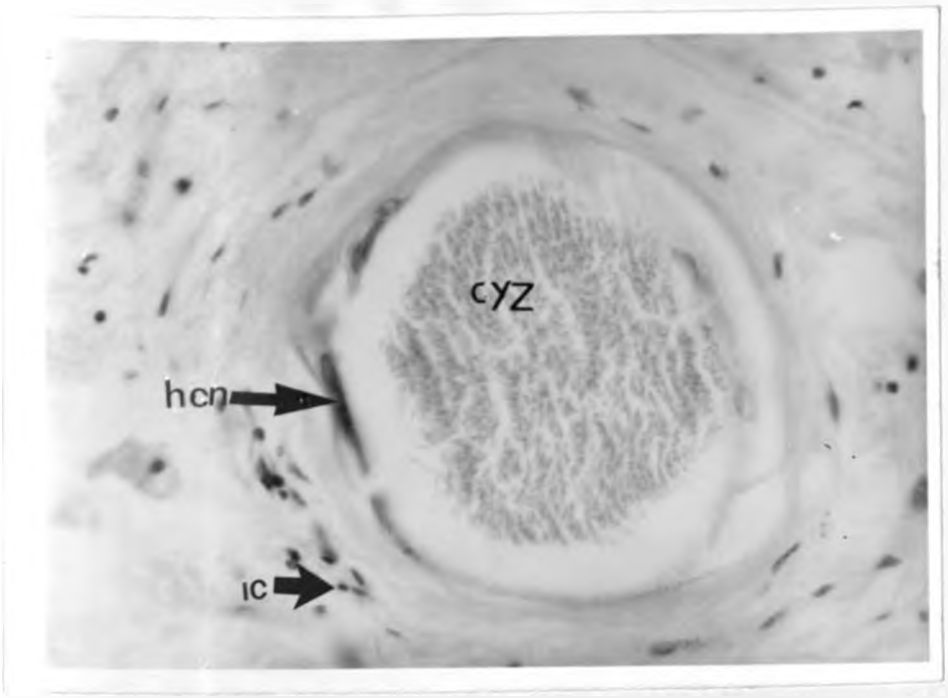
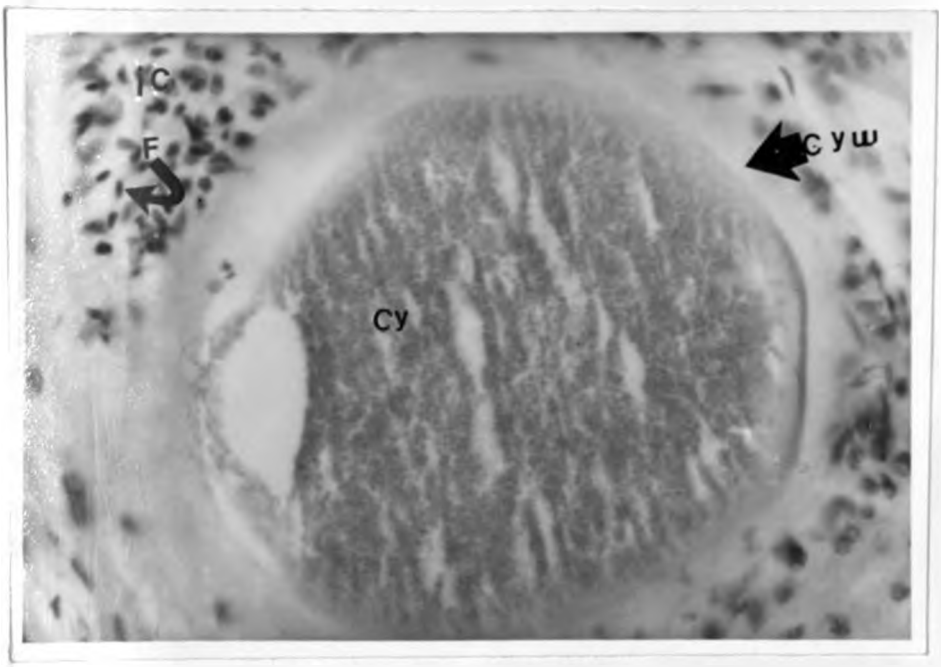
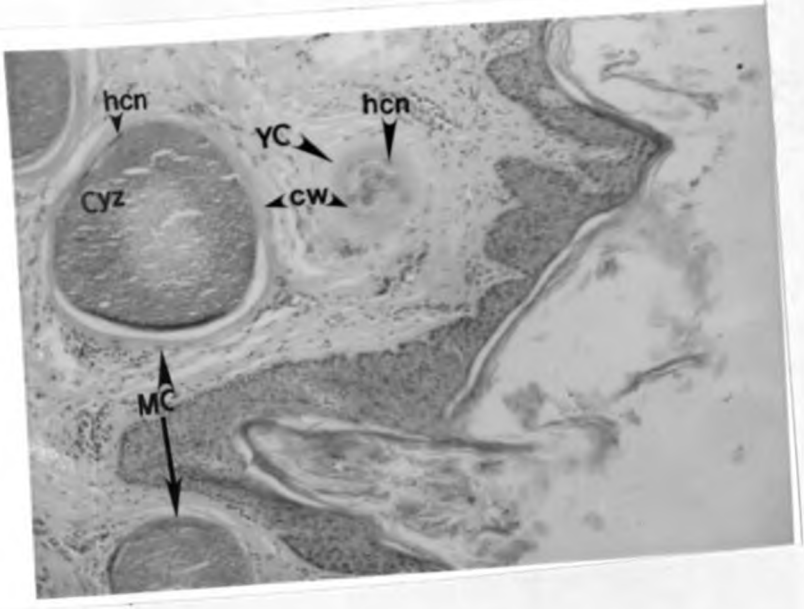


Figure 31

[31] A micrograph of a skin showing a large cyst with many highly granular cystozoites (cyz) which have pushed the host cell nuclei (hcn) towards the periphery and flattened them. Few inflammatory cells (ic) are visible. H&E X200 - 33 days post-inoculation.

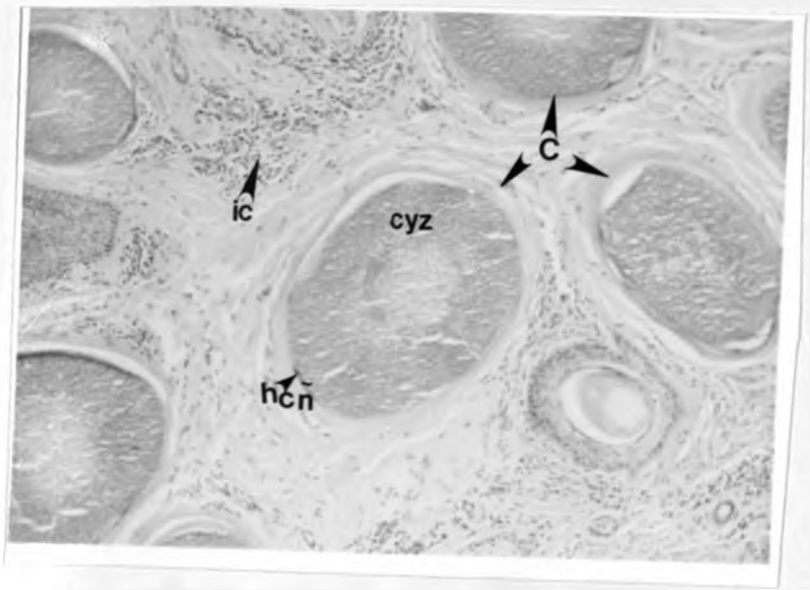


[32] Figure 32
A micrograph showing a large cyst with a thick cyst wall (CYW) and cystozoites (CYZ) arranged in stacks. The cyst is surrounded by fibroblasts (F) and inflammatory cells (ic) (H.&E. x 200, 40 days post-inoculation).



[33] Figure 33

A micrograph showing mature cysts (MC) with flattened host cell nuclei (hcn). A young cyst (YC) is also visible with round host cell nuclei. (H.&E. x 200, 56 days post-innoculation).



[34] Figure 34

A micrograph showing several mature cysts (C). The cysts have completely flattened host cell nuclei (hcn). The cystozoites (CyZ) appear dark staining on the periphery and light in the centre. Some inflammatory cells (ic) are present but the cysts themselves do not appear to have elicited specific inflammatory reactions around them. (H&E x200 - 66 days post-inoculation).

Day 112 (No. 306 killed): The clinical picture of the two remaining goats remained as they were on day 86. No bradyzoites were observed in their blood for the 26 days between day 86 and 112. Gross and histopathology remained as they were on day 86. The cysts were mainly mature but in very few instances immature cysts in any of the stages described previously were observed. The mean diameter of the mature cysts was 510µm.

Day 305 (No. 307 killed): The doe started showing reduction in number of cysts in the eye from day 120. Skin biopsy showed many of the mature cysts surrounded by inflammatory cells. By the sixth month, only two to three cysts could be seen in the eyes and the biopsies revealed very few cysts but showed many areas which had inflammatory cells. By the eighth month, all the cysts had disappeared and the inflammatory reactions that were there on the sixth month were not there.

No cysts were found in the doe that was killed on day 305. Either grossly or on histopathology.

4.9 Comparative ultrastructural studies on B. besnoiti and caprine besnoitia

4.9.1 Caprine besnoitia cysts

The outer cyst wall was thick (approximately 10µm) and was composed of collagen fibres that were mainly arranged concentrically (Figure 35). Between the collagen fibres there were many electron dense vesicles that varied greatly in their sizes and extended inwards except for a thin zone neighbouring the host cell. This

thin zone contained relatively homogenous tiny lightly electron dense particles.

The host cell cytoplasm had many short interdigitations with the thin outer cyst wall zone containing the relatively homogenous tiny lightly electron dense particles. The host cell cytoplasm composed of an electron lucent matrix interspaced with many tubular mitochondrion and tiny light and heavy electron dense particles that occurred singly in small or large clusters (Figure 35).

The host cell was multinucleate with the nuclei having one or more nucleoli. The nuclear membrane appeared as an electron dense single thick layer separating the chromatin and the cytoplasm. It was highly tortuous and contained indentations that had many mitochondrion.

The chromatin of the host cell nuclei was composed of an electron lucent matrix interspaced with loosely packed light and heavily electron dense particles and one or more nucleoli (Figure 35).

The host cell cytoplasm was separated from the cyst vacuole by a vacuole membrane. Originating tangentially or perpendicularly from the vacuole membrane were strands of closely packed light electron dense granules that formed the inner cyst wall. High magnification revealed that the strands were many minute vesicles which were closely packed just below the vacuole membrane and decreased inwards and terminated inside the cyst vacuole (Figure 35).

Immediately below and very close to the inner cyst wall, cystozoites mainly with their anterior end pointing outwards and many ribosome-like structures were observed.

The cyst vacuole matrix was eletron lucent and contained the cystozoites, ribosome-like structures and very fine light electron dense particles. In some cysts, especially the old ones, dead cystozoite debris were observed in the matrix in the middle of the cyst (Figures 35,36).

4.9.2 Besnoitia besnoiti cysts

The outer cyst wall was very thick (approximately 30µm) and was composed of collagen fibres that were mainly arranged concentrically (Figure 37). Between the collagen fibres there were many fine uniformly sized heavily electron dense particles that extended inwards except for a thin zone next to the host cell. This zone contained relatively homogenous tiny lightly electron dense particles and lipid vacuoles (Figure 37).

The host cell cytoplasm had many long interdigitations with the thin zone containing the tiny lightly electron dense particles and lipid vacuoles of the outer cyst wall. In some cysts, many lipid vacuoles were found between the host cytoplasm and the collagen and also inside the cytoplasm (Figure 37).

The matrix of the host cell cytoplasm was made of fine light electron dense material. There were dense rough endoplasmic reticulum (RER) and very few mitochondria. There were also many fine heavily electron

The cyst vacuole matrix was electron lucent and contained the cystozoites, ribosome-like structures and very fine light electron dense particles. Cystozoite debris was also visible throughout within the cyst vacuole (Figure 37).


N
W2
NU
CYZ
id
hcg
B
C
D
1.0μm

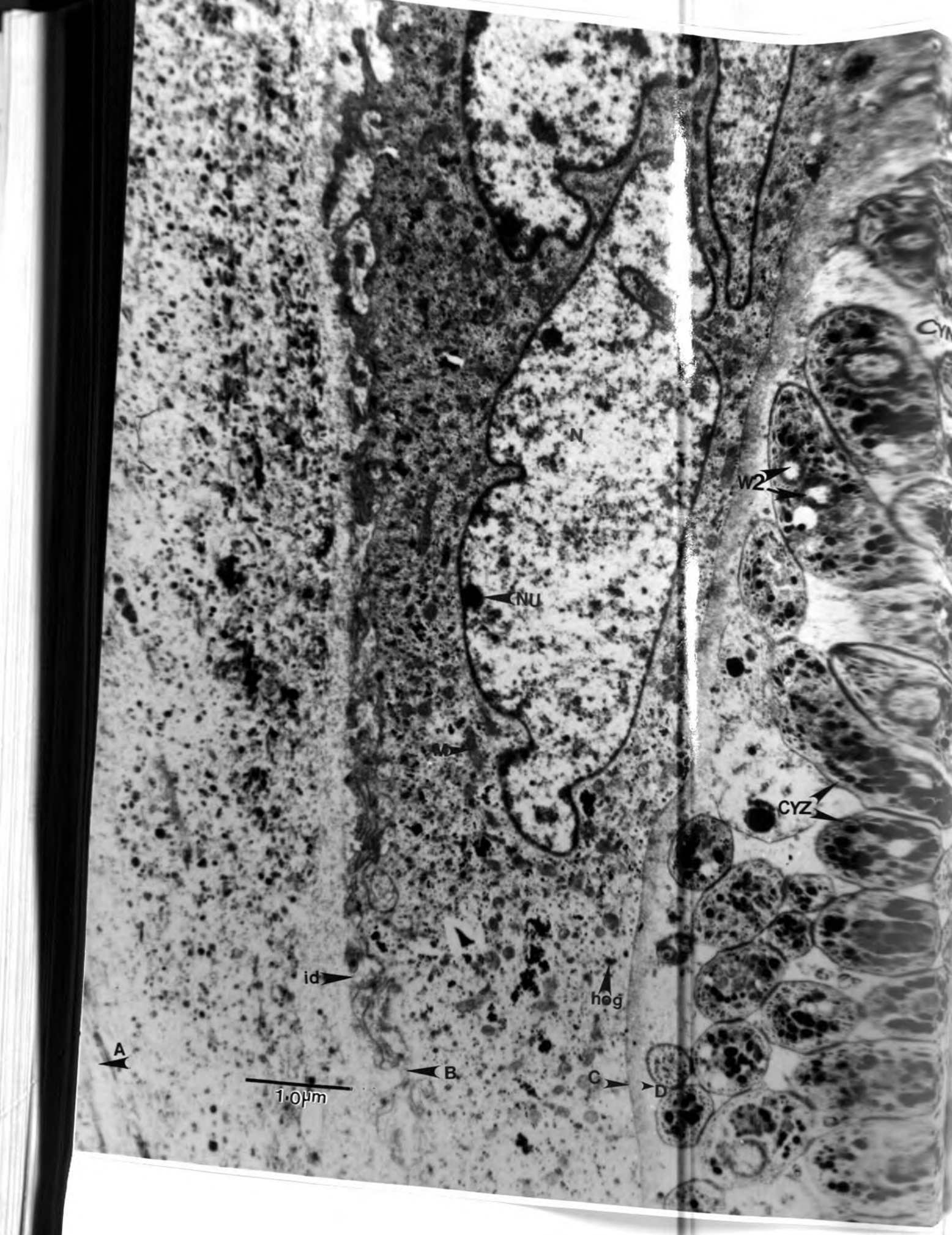

N
NU
W2
CYZ
id
hog
A
B
C
D
1.0μm

Figure 35

An electron micrograph of a section of a caprine besnoitia cyst showing cystozoites (CYZ) inside the parasitophorous vacuole (PV), the vacuole membrane (VM) from which strands of lightly electron dense granules which are attached tangentially or longitudinally to form the inner cysts wall (between c and d), the multinucleate host cell (between b and c) with large number of tubular mitochondrion (M), the nucleus (N) have one or more nucleoli (Nu), the host cell interdigitate (ID) with the collagenous outer cyst wall (between A and B).

Note: The mainly anterior direction of top cystozoite towards the inner cysts wall, the presence of more than one wall forming body two (W2) in one cystozoite. The W2 are in between the cystozoite nucleus (cyn) and the anterior end of the cystozoite. Note also the great variation in sizes of particles or granules in the outer cyst wall and presence of the fine heavily electron dense granules (hog) in the host cell cytoplasm. (Uranyl acetate x 8820)

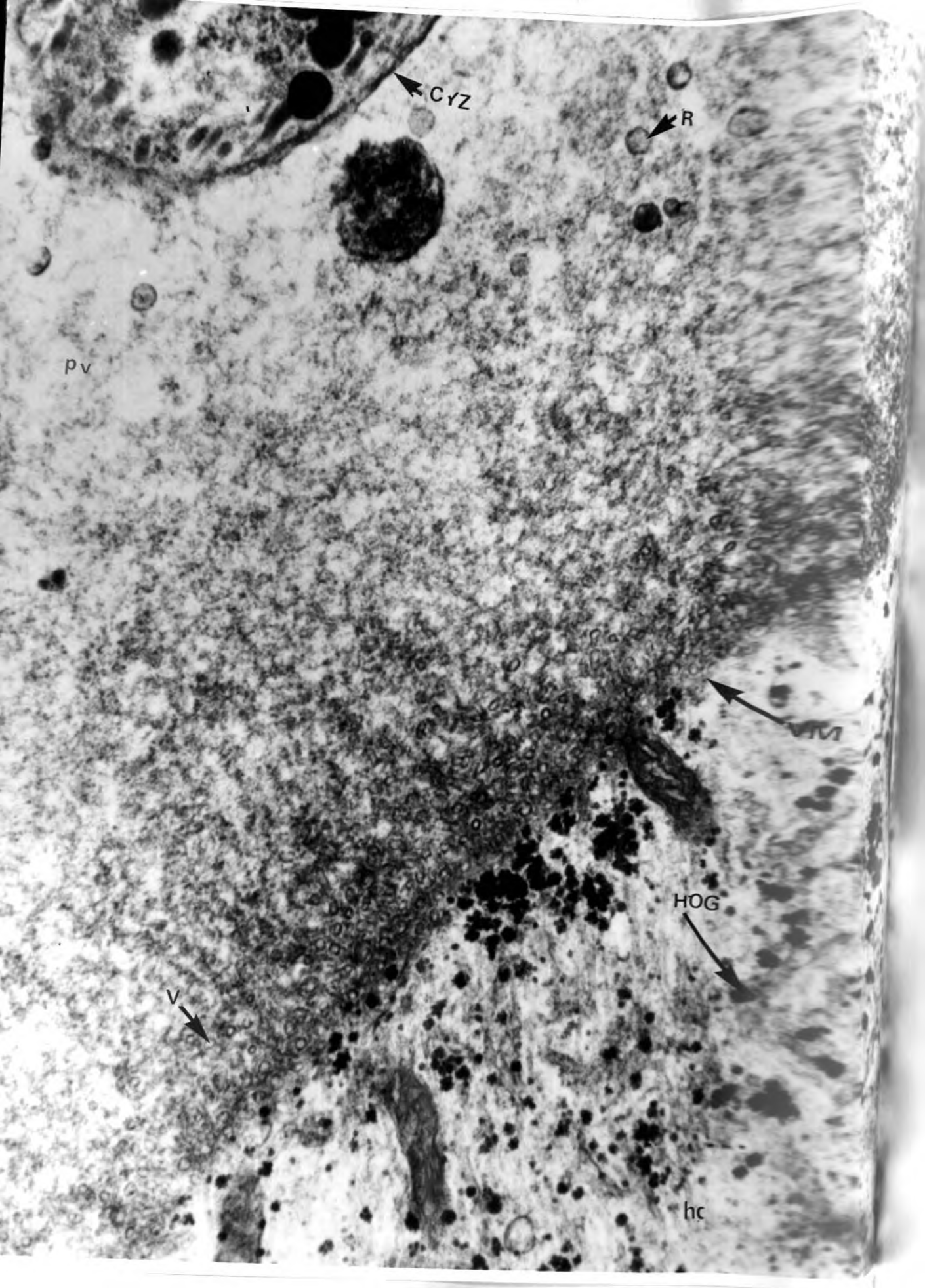

CYZ
R
pv
VM
HOG
V
hc

Figure 36

A high power section showing a section between the host cell (hc) and the parasitophorous vacuole (PV) of a caprine besnoitia cyst. The vacuole membrane (VM) with many vesicles (V) and fine particles form the inner cyst wall. The host cell has heavily electron dense granules (HoG), while ribosome-like structures (R) and a cystozoite (CyZ) can be seen in the PV. Uranyl acetate x56000.

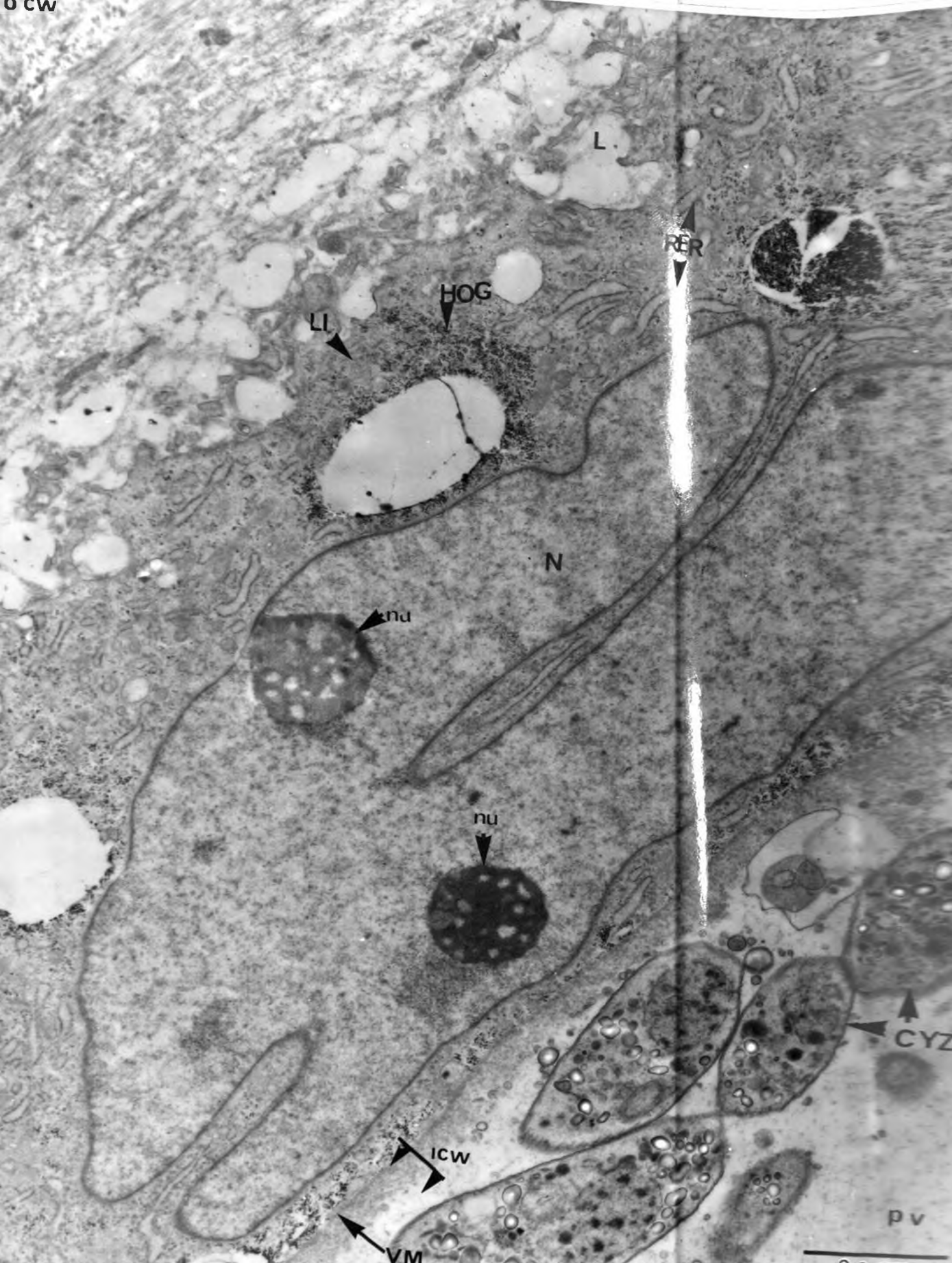

6 cw
L
RER
HOG
LI
N
nu
nu
CYZ
icw
VM
pv

Figure 37

A medium power electron micrograph of a section of B. besnoiti cyst showing cystozoites (CYZ) inside a parasitophorous vacuole (PV). The vacuole membrane (Vm) from which light electron dense granules attach inwards to form the inner cyst wall (ICW). The host cell (HC) with the highly indented nucleus (N) with two vacuolated nucleoli (nu), a cytoplasm with a lot of rough endoplasmic reticulum ((RER) and many heavily electron dense granules (HOG) especially bordering the vacuole membrane as well as lightly electron dense (Li) granules and ribosome-like structures (R) are present. The collagenous outer cyst wall (OCW) is divided with the host cell by a vacuolated lipid (L) containing layer.

Note: The light electron dense matrix of the nucleus of the HC and the clear double membrane of the cystozoite nucleus (Cn). Uranyl acetate x 11200.

4.9.3 Cystozoites of B. besnoiti and caprine besnoitia

Cystozoites of both caprine besnoitia and Besnoitia besnoiti were crescent shaped and bound by a three layered pellicle. The pellicle was on average 30 nm in diameter in caprine besnoitia and ranged from 20-40 nm in B. besnoiti. The pellicle of caprine besnoitia was smooth while that of B. besnoiti was wavy and the outer membrane appeared to form junctions with itself and with the inner membrane (Figures 38 and 43).

The outer membrane of the pellicle was continuous and enveloped the whole organism in the two besnoitia species. The inner membrane and the outer membrane were separated by an electron lucent space which was on average 10nm wide in caprine besnoitia and ranged from 10-30nm in B. besnoiti. The inner membrane was continuous for most parts of the organism except where the outer membrane invaginated to form micropore and anteriorly where it thickened and terminated at the same level as the anterior conoid.

The micropore was formed in both besnoitia species by the invagination of the outer membrane of the pellicle. In caprine besnoitia the width at the opening was on average 60-70nm, the narrowest part of the neck was 40nm, the widest part of the micropore 120nm, the depth was 170nm, the outer cylinder had a diameter of 150nm and a height of 80nm. In B. besnoiti the opening was 50-60nm, the narrowest part of the neck was 40nm, the widest part of the micropore was 80nm and the depth was 180nm. The outer cylinder of the micropore in caprine

besnoitia was formed from the thickening of the inner membrane of the pellicle and an outgrowth from the outer membrane of the pellicle at the point where invagination starts. In B. besnoiti the cylinder was formed just by the thickening of the inner membrane of the pellicle which begins well before the point of invagination. In both species the cylindrical structure lining the micropore terminated where the hemisphere of the micropore widened out fully (Figures 38,41,42,44,47,48).

The anterior end of the two besnoitia species were typical of the apicomplexa. They had a conoid, ducts of rhoptries, microtubules, micronemes and polar ring. The conoid was cone-shaped with fibrillar structures arranged in a spiral. The diameter of the base of the cone was an average 300-310nm, while the narrow end (anterior) was 140-160nm in diameter. The height of the cone in caprine besnoitia was on average 280nm and 230nm in B. besnoiti. The conoid in both organisms was seen either above, below or at the same level with the polar ring (Figures 38,39,40,43,44,46).

The ducts of rhoptries in the two species originated from within the conoid. They were long club-like electron dense structures with a diameter ranging from 30nm to 120nm at the narrowest and widest respectively. Longitudinal sections of the cystozoites showed the ducts of rhoptries originating at one or two points within the conoid in both B. besnoiti and caprine besnoitia. In both organisms there were three pairs of ducts of rhoptries (Figures 39,40,43,46).

There were twenty two regularly arranged microtubules in both species that originated from the polar ring. They were on average 40nm in diameter in both species. They had an electron dense outer part and an electron lucent centre. Close observation of the microtubules on the longitudinal, transverse and tangential sections of cystozoites of the two organisms showed a marked difference in the direction of the microtubules. In Besnoitia besnoiti the microtubules after originating from the polar ring go straight posteriorly approximately for the first one sixth of the cystozoite after which they spiral and terminate on the posterior pole of the cystozoite. In caprine besnoitia the microtubules after originating from the polar ring remain straight all the way to the posterior end of the cystozoite (Figures 38,39,40,41,43,44,47).

Micronemes which were electron dense like rhoptries were found in large numbers in most parts of the cystoplasm. They were elongated oval in shape with largely varied length 60-250nm and a mean diameter of 40nm in both besnoitia species.

Inside the cytoplasm of some of the cystozoites one or two different wall forming bodies (dense bodies) were seen. The dark dense body was designated wall forming body one (W1) and the larger spongy honey comb looking like body was designated wall forming body two (W2).

In caprine besnoitia the W1 was a membrane bound dark, dense, homogenous body that was always found very close to the side or anterior to the nucleus. It was

usually round with a diameter of .6-1.2µm. The W1 in caprine besnoitia appears to transform to W2. It loses its limiting membrane, shrinks and changes to a larger spongy round or oval shaped membrane bound by a lighter body (W2). The internal appearance of is that of a bee-comb. As the W2 enlarges to diameters ranging from 0.7 to 1.6µm it migrates anteriorly and away from the nucleus to rest half-way between the latter and the anterior end of the cystozoite. At this time no traces of W1 were seen in the cystozoites. In a few cases two W2 bodies were seen in one cystozoite (Figures 38,39,40).

In B. besnoiti W1 was a membrane bound round body containing many small membrane bound bodies in it. The closer the small bodies were packed the darker the W1 appeared. The W1 ranged from 0.3 -0.6µm in diameter. In B. besnoiti the W1 and W2 were at times found in the same cystozoite on the opposite side of the nucleus. The W2 in B. besnoiti was similar in appearance and shape with W2 of caprine besnoitia and was also found halfway between the nucleus and the anterior end of the cystozoite. W2 in B. besnoiti had a diameter of 0.6-0.7µm and in one cystozoite there was evidence that it was undergoing fusion or division (Figures 43,44,45).

Amylopectin and lipid granules were found in the cytoplasm of the cystozoites of both organisms. Caprine besnoitia appeared to have more amylopectin represented by heavily electron dense dense homogenous bodies, than B. besnoiti. However B. besnoiti appeared to have more lipid represented by empty vacuoles which were at times

ringed with an electron dense zone than caprine besnoitia (Figures 38,40,41,43,46,47).

The nucleus of both besnoitia species were either oval or gourd shaped and was found lying below the half mark of the cystozoite posteriorly. In caprine besnoitia it was always surrounded by a halo measuring on average 40nm. In B. besnoiti the nuclear membrane was found intact in most cystozoites and measured on average 30nm. The chromatin in caprine besnoitia appeared relatively homogenous as opposed to that of B. besnoiti which appeared granular (Figures 38,39,41,43,46,47).

Other organelles that were observed in the cytoplasm were mitochondria and ribosome-like structures. The mitochondria and ribosome-like structures were more readily demonstrable in B. besnoiti than in caprine besnoitia. The ribosome-like structures occurred either as single double membrane bound structures, or in twos or as clusters surrounded by a membrane. In addition some ribosome like structures were found being released from the anterior part of some cystozoites into the cyst fluid by exocytosis (Figures 40,43,45,46)

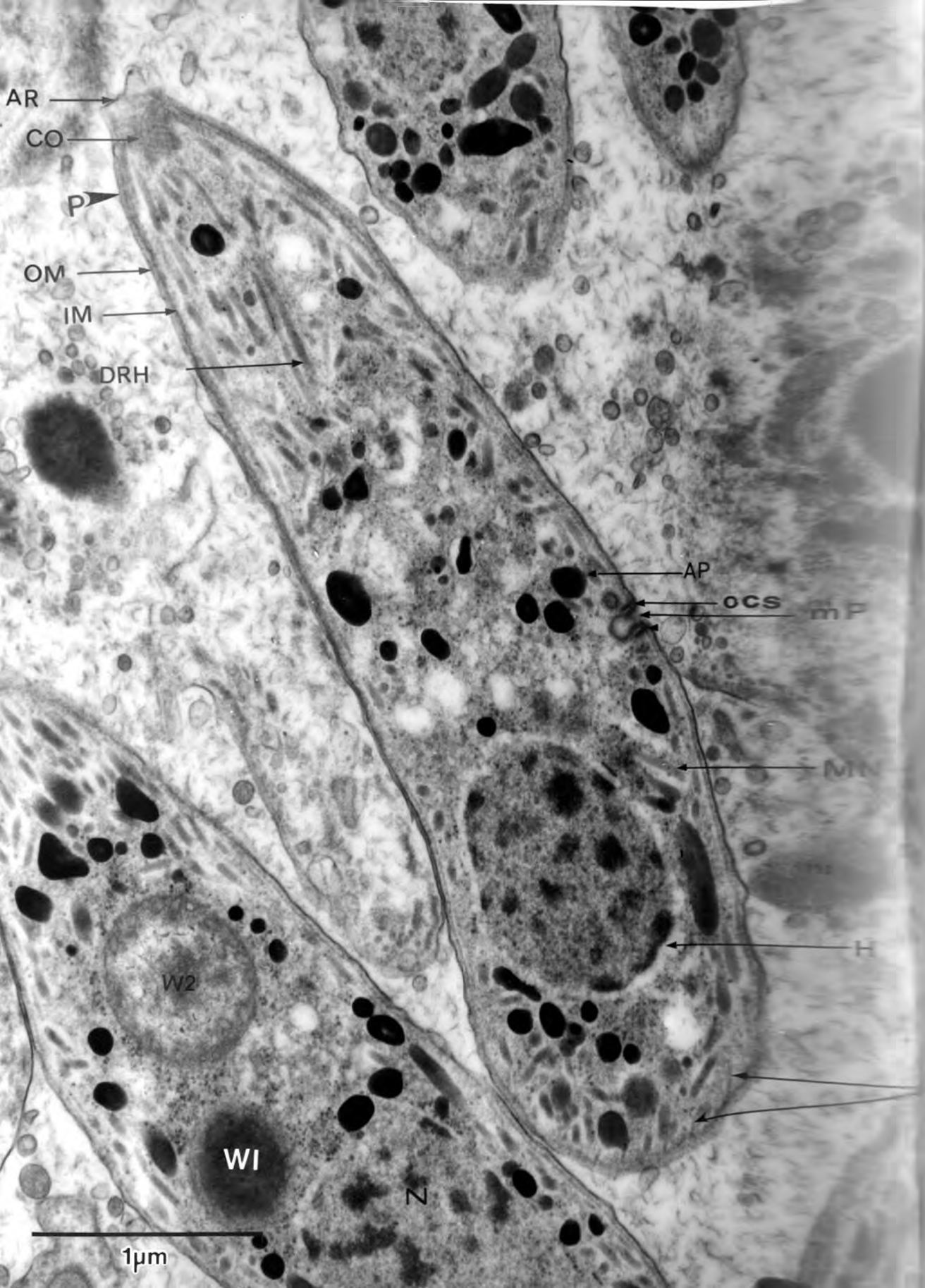

AR
CO
P
OM
IM
DRH
AP
ocs
mP
MN
H
W2
WI
N
1µm

Figure 38

An electron micrograph showing longitudinal and tangential sections of cystozoites of caprine besnoitia showing the pellicle (P), the conoid (CO), the long club-like ducts of rhoptries (DRH), the short club-like micronemes (MN), the amylopectin granules (AP), the micropore (MP), the nucleus (N) surrounded by a halo zone (H), the microtubules (MT) and the wall forming bodies one (W1) and two (W2).

Note: The crescent shape of the complete organism, the smooth appearance of the pellicle, the spiral-fibrillar structure of the conoid, the electron dense nature of the ducts of rhoptries, micronemes and amylopectin, the parallel arrangement of the microtubules, the electron dense, dense homogenous nature of W1 and its closeness to the nucleus and the spongy honey-comb internal appearance of W2. the complete organism neither has the W1 or W2 (70 days post-inoculation). (x36400).

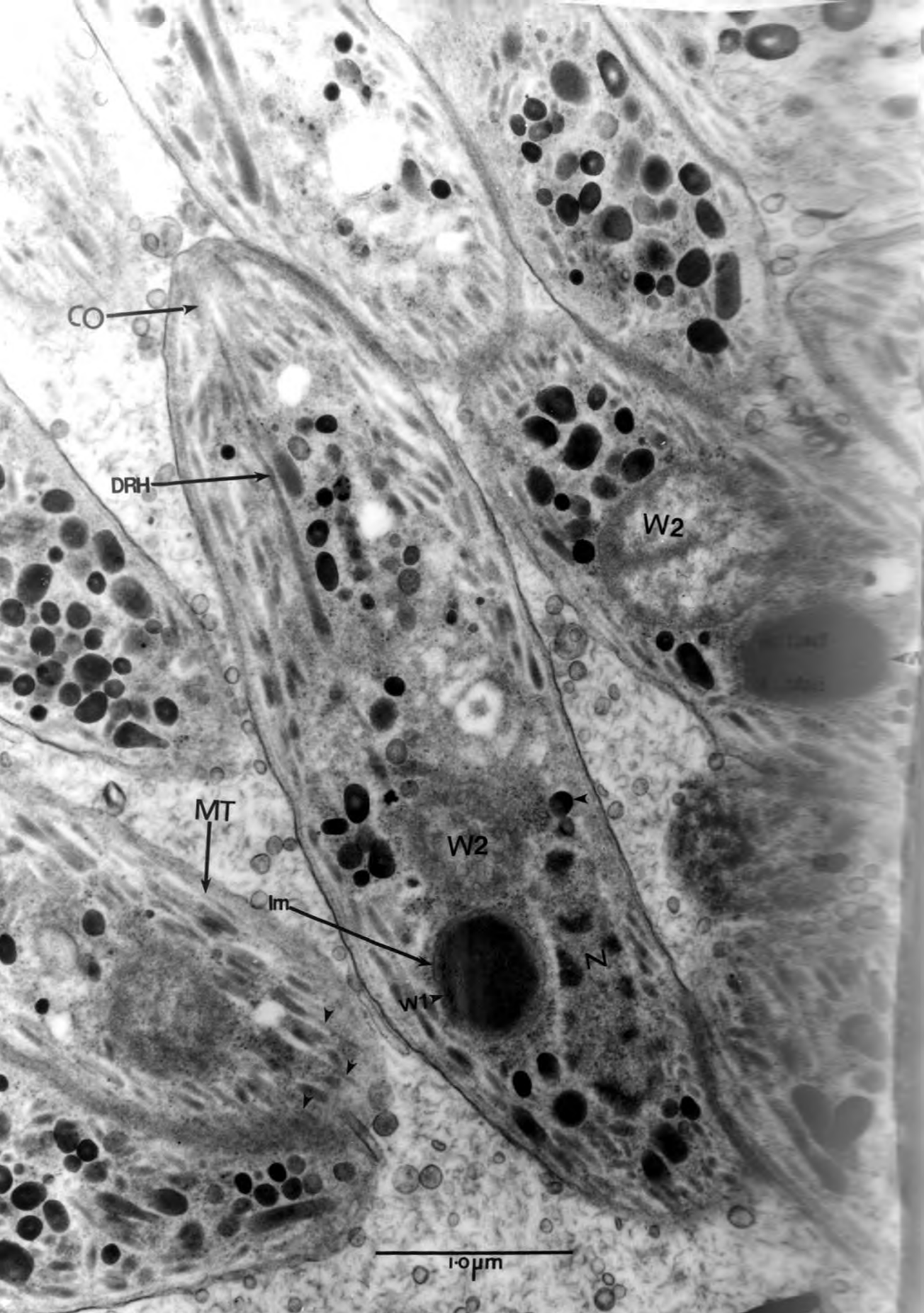

CO
DRH
W2
MT
W2
Im
N
W1
1·0 µm

Figure 39

An electron micrograph showing longitudinal and tangential sections of cystozoites of caprine besnoitia showing the smooth pellicle (P), conoid (CO), ducts of rhoptries (DRH), the microtubules (MT), amylopectin granules (AP), the wall forming bodies 1 (W1) and two (W2), the micronemes (MN) and the nucleus (N).

Note: Parallel straight nature of the microtubules (arrows), the wall forming body 1 (W1) on the left cystozoite has a limiting membrane and the wall forming body two (W2) on the same cystozoite does not have the limiting membrane (Lm) while in the cystozoite on the right, W1 has shrank and lost its limiting membrane as the W2 has enlarged and developed a limiting membrane. Note also the high concentration of micronemes (MN) in all the cystozoites and close proximity of the W1 on the left with the nucleus. Uranyl acetate x 36400.

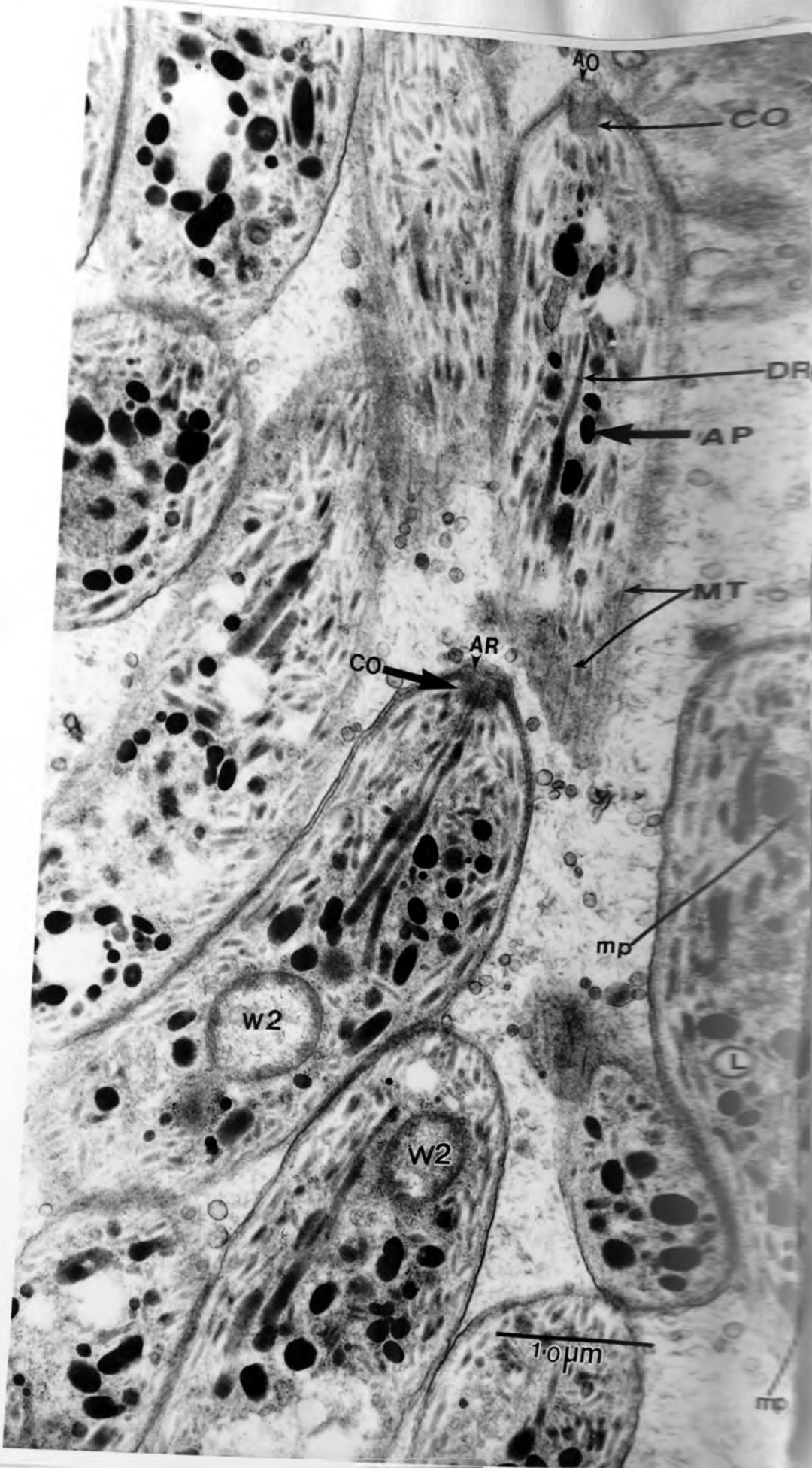

AO
CO
DR
AP
MT
AR
CO
mp
W2
W2
L
1·0 μm

Figure 40

An electron micrograph showing cystozoites of caprine besnoitia that have been sectioned just below the pellicle (P).

Note: The parallel straight microtubules (MT), the orifice of the rhoptries (AO), the conoid (CO), the duct of rhoptries (DRH) the apical ring (AR), the wall forming bodies two (W2) and transverse sections of two micropores (MP). (Uranyl acetate x25000)

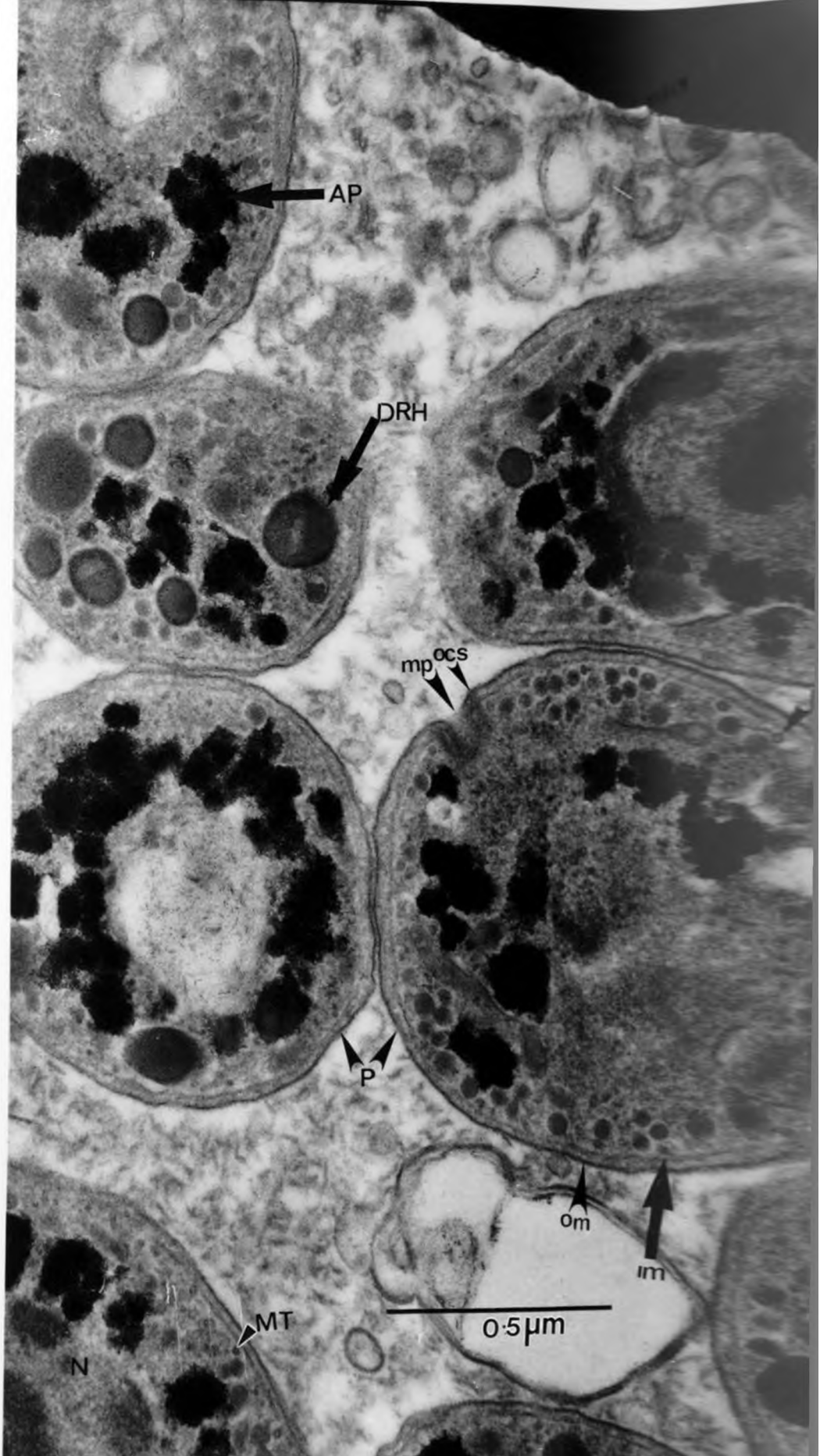
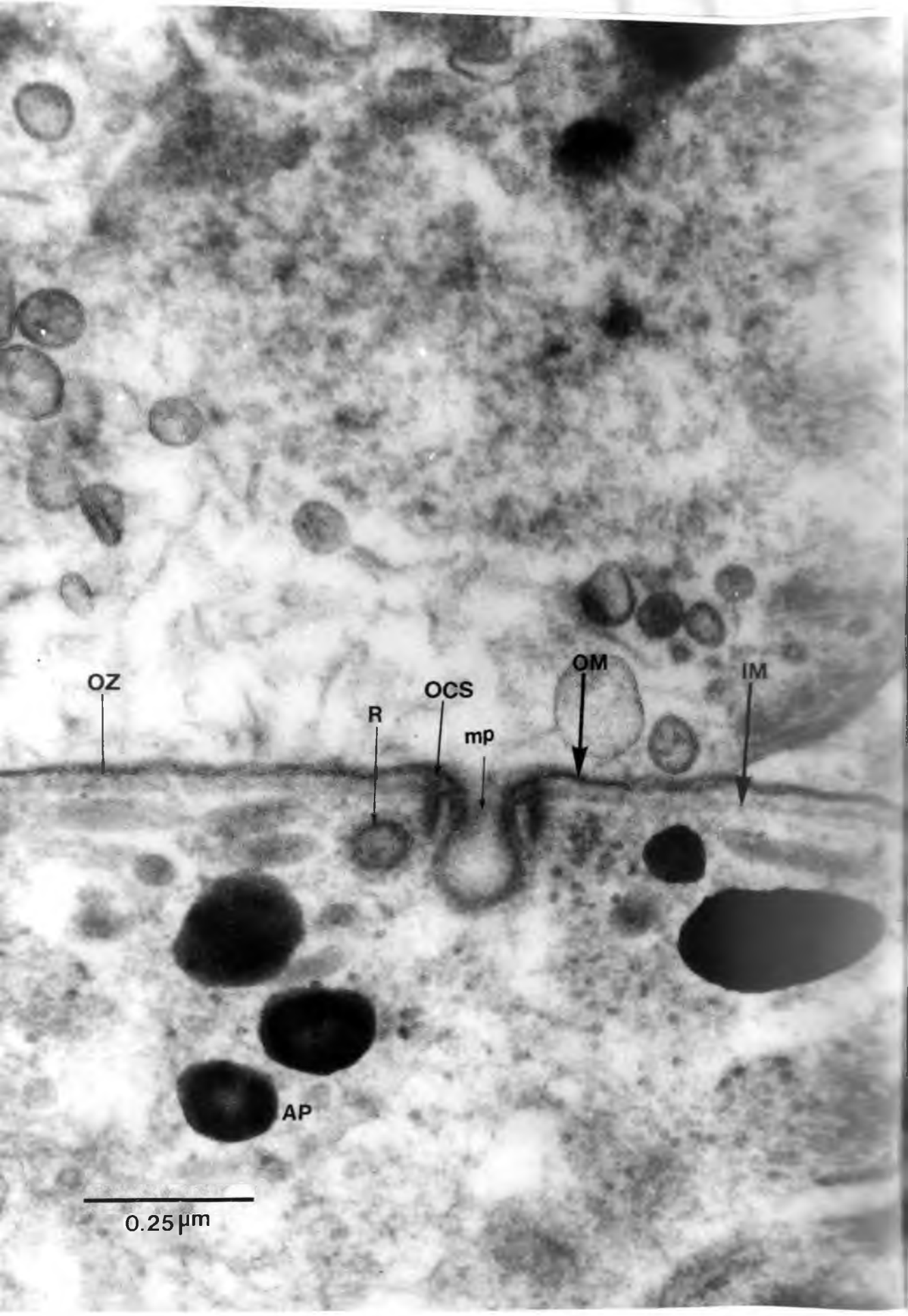

AP
DRH
mp
ocs
P
om
im
MT
N
0·5 µm

Figure 41

An electron micrograph showing transverse and tangetial sections of caprine besnoitia cystozoites.

Note: The smooth pellicle (P) with its inner (im) and outer (om) membranes, the hollow parallel evenly spaced microtubules (MT), the micropore (MP) with the outer cylindrical structure (OCS) formed by both the im and om, a cystozoite with the six ducts of rhoptries (DRH), large numbers of micronemes (MN) the nucleus (N) and amylopectin (AP) granules. Uranyl acetate x70000.


OZ
R
OCS
mp
OM
IM
AP
0.25 µm

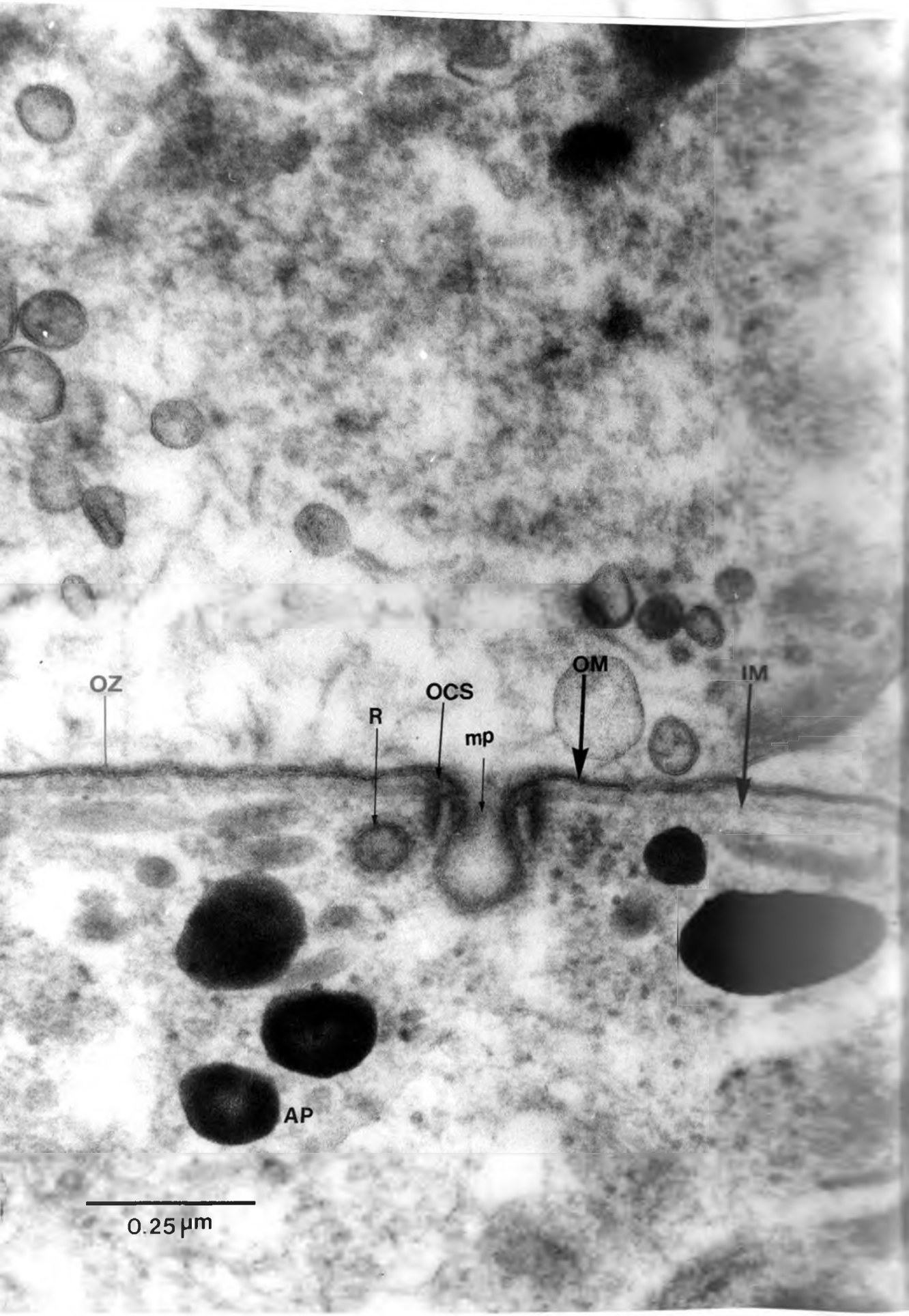

OZ
R
OCS
mp
OM
IM
AP
0.25 µm

Figure 42

An electron micrograph showing the fine details of a longitudinal section of the micropore (MP) of caprine besnoitia.

Note: The outer cylindrical structure (OCS) of the micropore appear to be formed from the outer (OM) and inner (IM) membranes of the pellicle. The electron lucent zone (OZ) separating the two membranes is even. Ribosome -like structures (R) are found in the cytoplasm of the cystozoite and free in the cyst fluid. Amylopectin (AP) granules are also seen in this section. Uranyl acetate x145600.

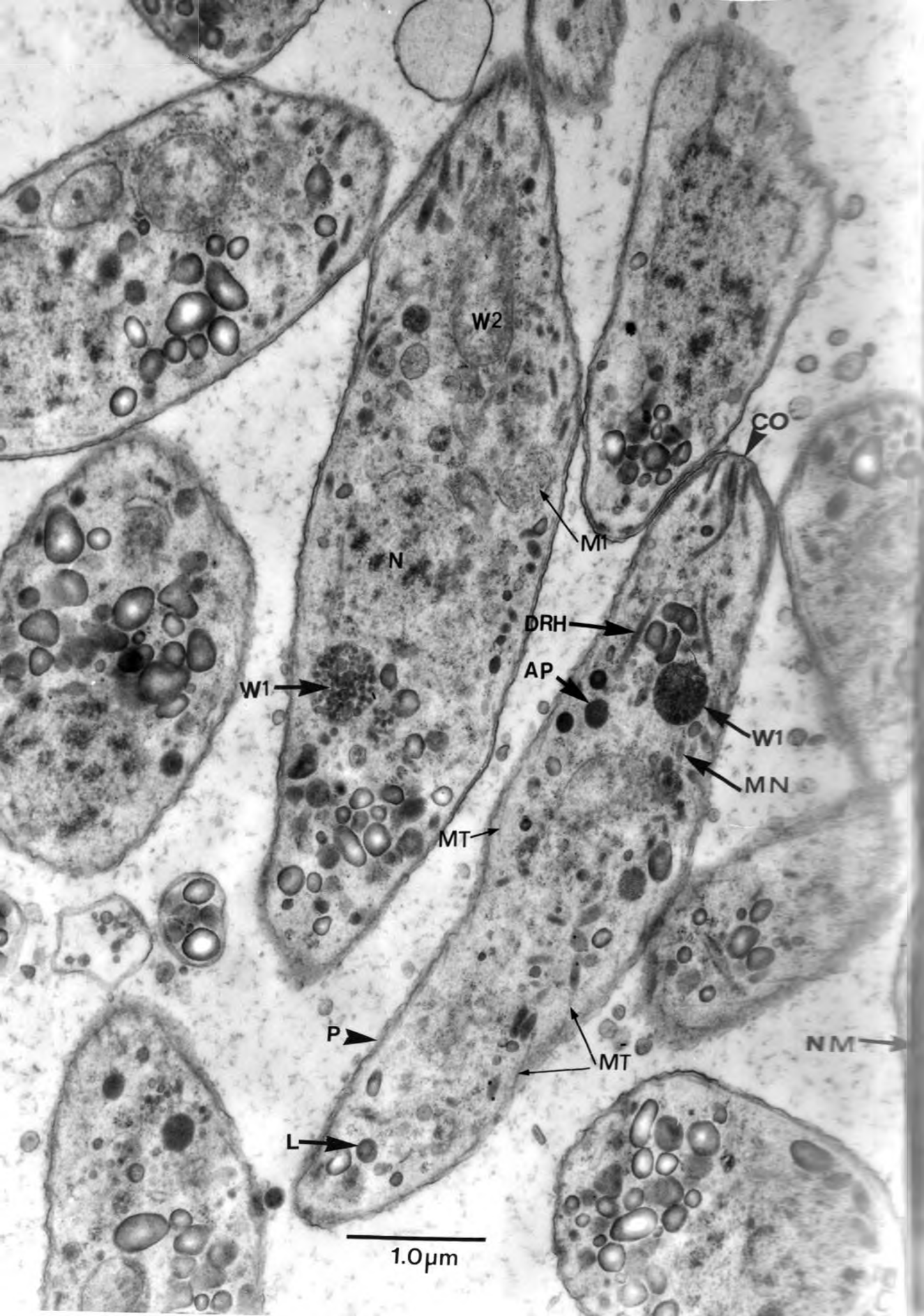

W2
CO
M1
N
DRH
AP
W1
W1
MN
MT
P
MT
NM
L
1.0μm

Figure 43

An electron micrograph showing longitudinal and tangential sections of cystozoites of Besnoitia besnoiti showing the pellicle (P), the conoid (CO), the long club-like ducts of rhoptries (DRH) originating from within the conoid, the short club-like micronemes (MN), the amylopectin (AP) and lipid granules (L), the mitochondrion (MI), the nucleus (N) with or without the nuclear membrane (NM), the microtubules (MT) and the wall forming bodies one (W1) and two (W2).

Note: The crescent shape of the complete organism, the wavy appearance of the pellicle, the spiral-fibrillar structure of the conoid, the electron dense nature of the ducts of rhoptries, the oblique (spiral) arrangement of the microtubules. The W1 is membrane bound and has small membrane bound electron dense dense bodies in it. The W2 has a spongy internal appearance (70 days post-inoculation). (Uranyl acetate x26900)

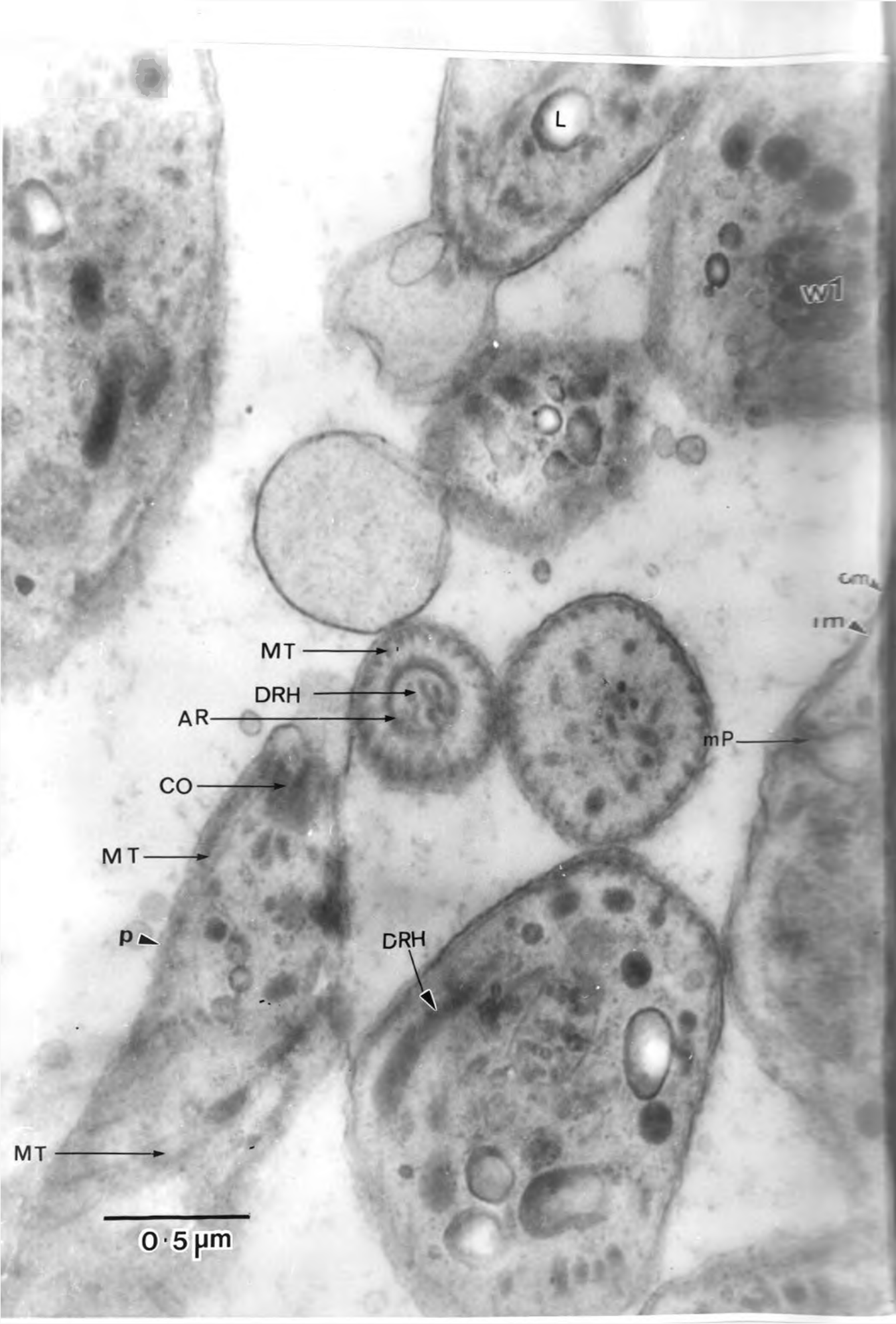
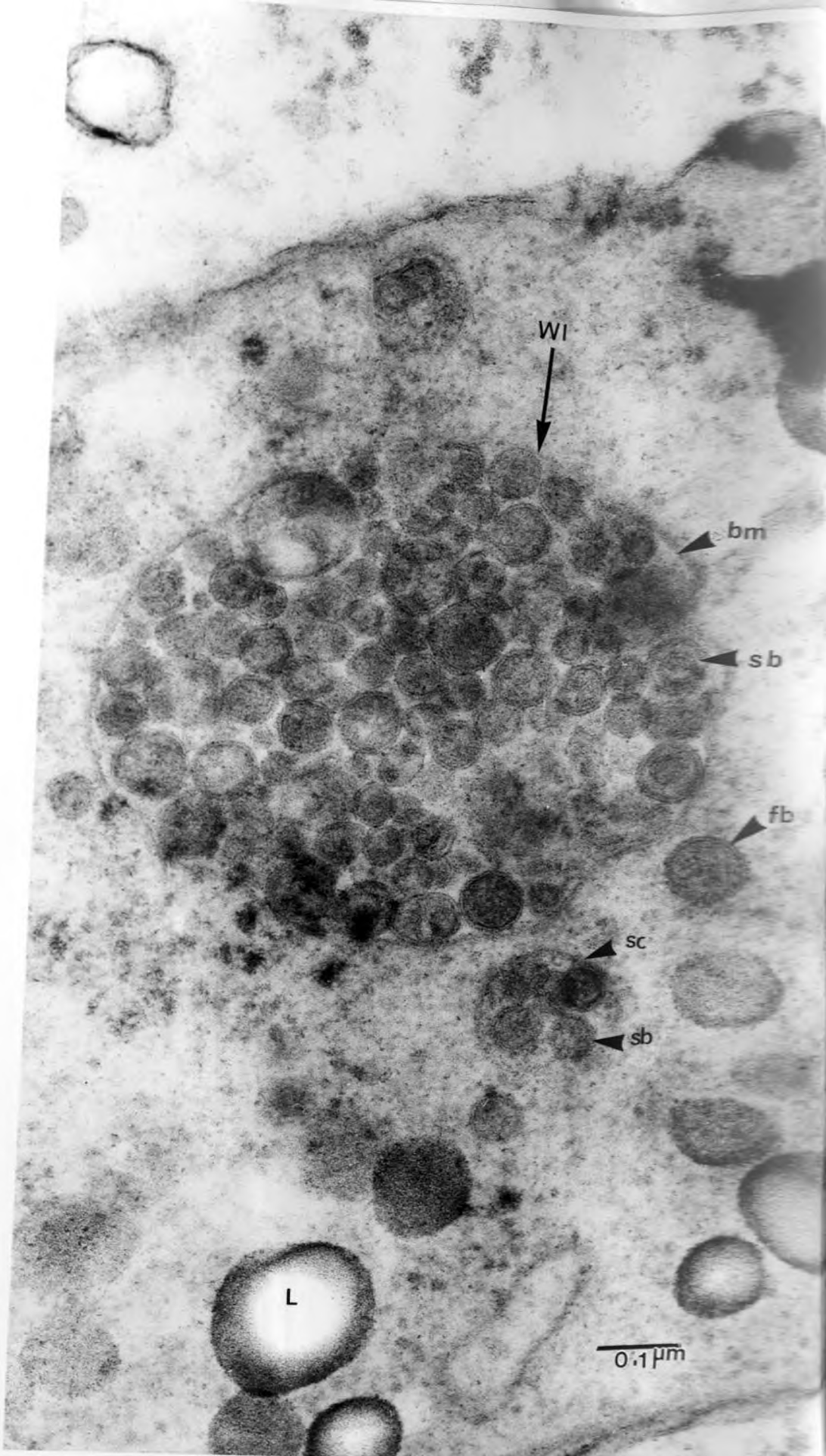

L
W1
cm
im
MT
DRH
AR
mP
CO
MT
p
DRH
MT
0·5 µm

Figure 44

An electron micrograph showing longitudinal, transverse and tangential sections of B. besnoiti.

Note: The conoid (CO) both on transverse and longitudinal sections surrounded by the apical ring (AR),, the wavy nature of the pellicle (P), the micropore (MP) formed by the outer membrane (OM) and the inner membrane (IM). The regularly arranged microtubules (MT) are straight on the anterior end but spiral posteriorly as seen in the cystozoite on the left. Ducts of rhoptries (DRH), an early wall forming body 1 (W1), lipids (L) and amylopectin granules (AP) are also present. (Uranyl acetate x70000)


WI
bm
sb
fb
sc
sb
L
0.1 µm

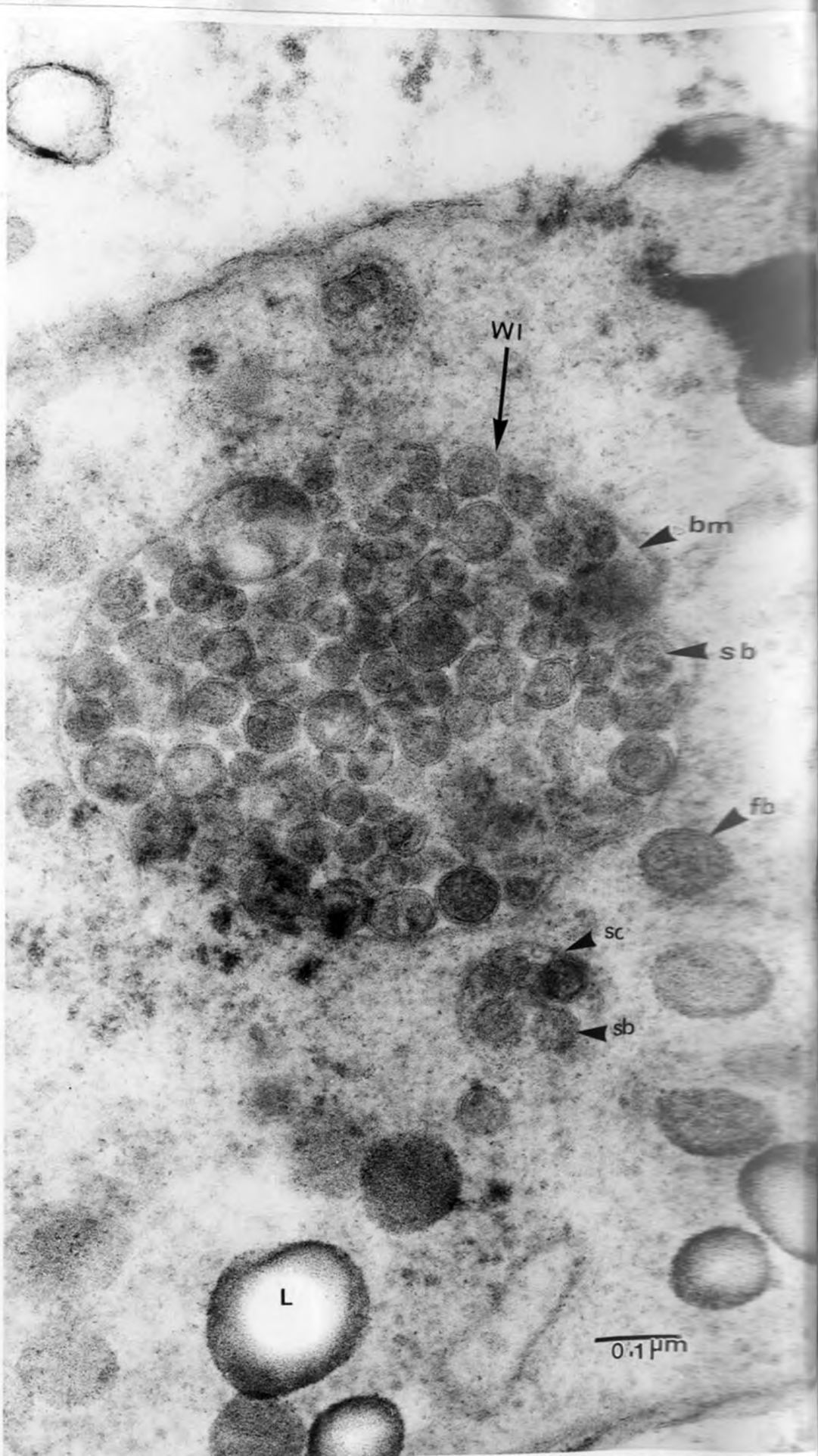

WI
bm
sb
fb
sc
sb
L
0.1 µm

Figure 45

An electron micrograph of Besnoitia besnoiti cystozoite showing the fine details of wall forming body one (Wl). Note its small bodies (Sb) inside the body membrane (bm). Other small bodies which are free (fb) or in small clusters (SC), and lipids are present. Uranyl acetate x176000.

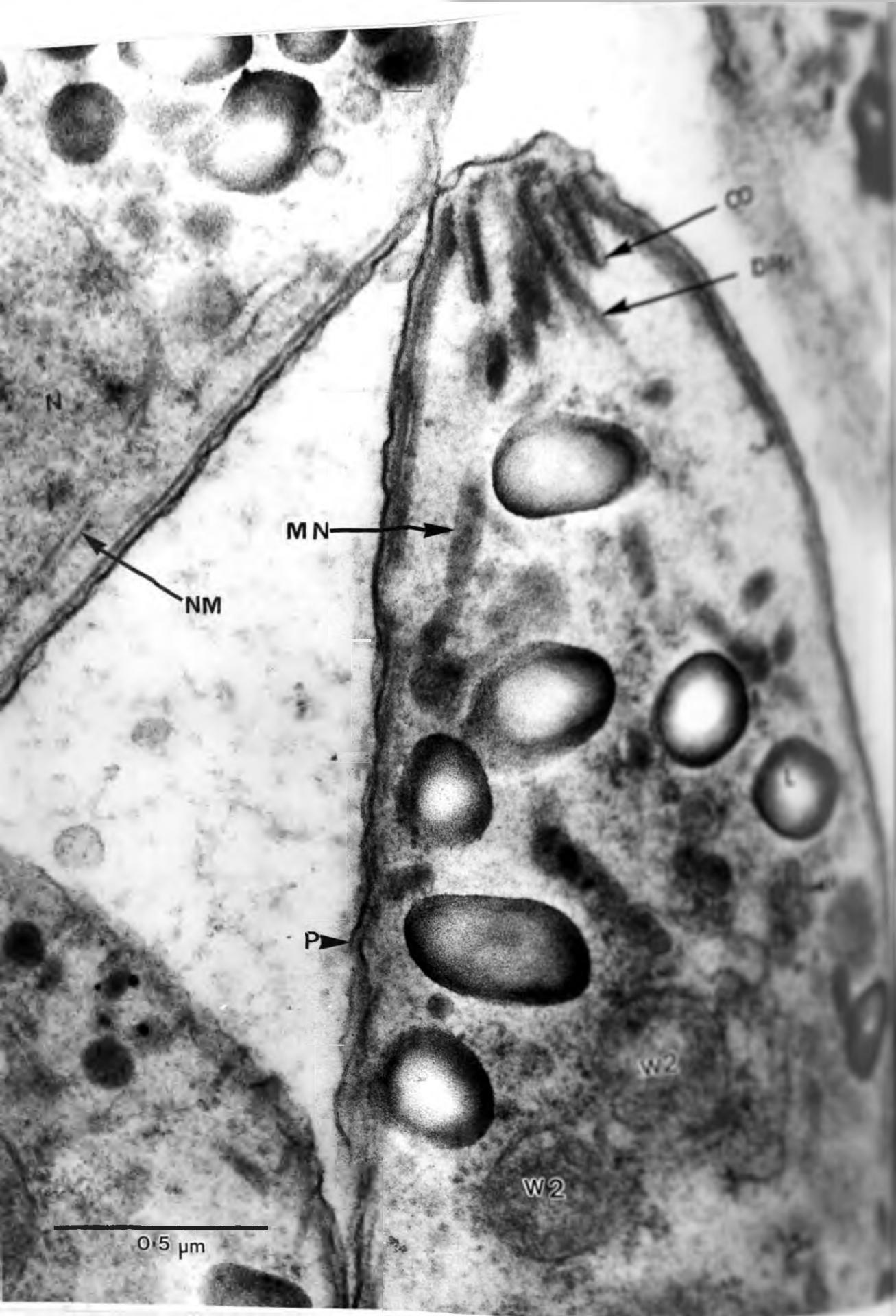

CD
DM
N
MN
NM
P
W2
W2
0·5 µm

Figure 46

A micrograph showing a longitudinal and tangential sections of cystozoites of B. besnoiti.

Note: The wall forming body 2 (W2) seem to be dividing or fusing, the ducts of rhoptries (DRH) originating inside the conoid (CO), the lipid granules (L), micronemes (MN), ribosome-like structures (R), the wavy pellicle (P) and the nucleus (N) with nuclear membrane (NM). (Uranyl acetate x70000)

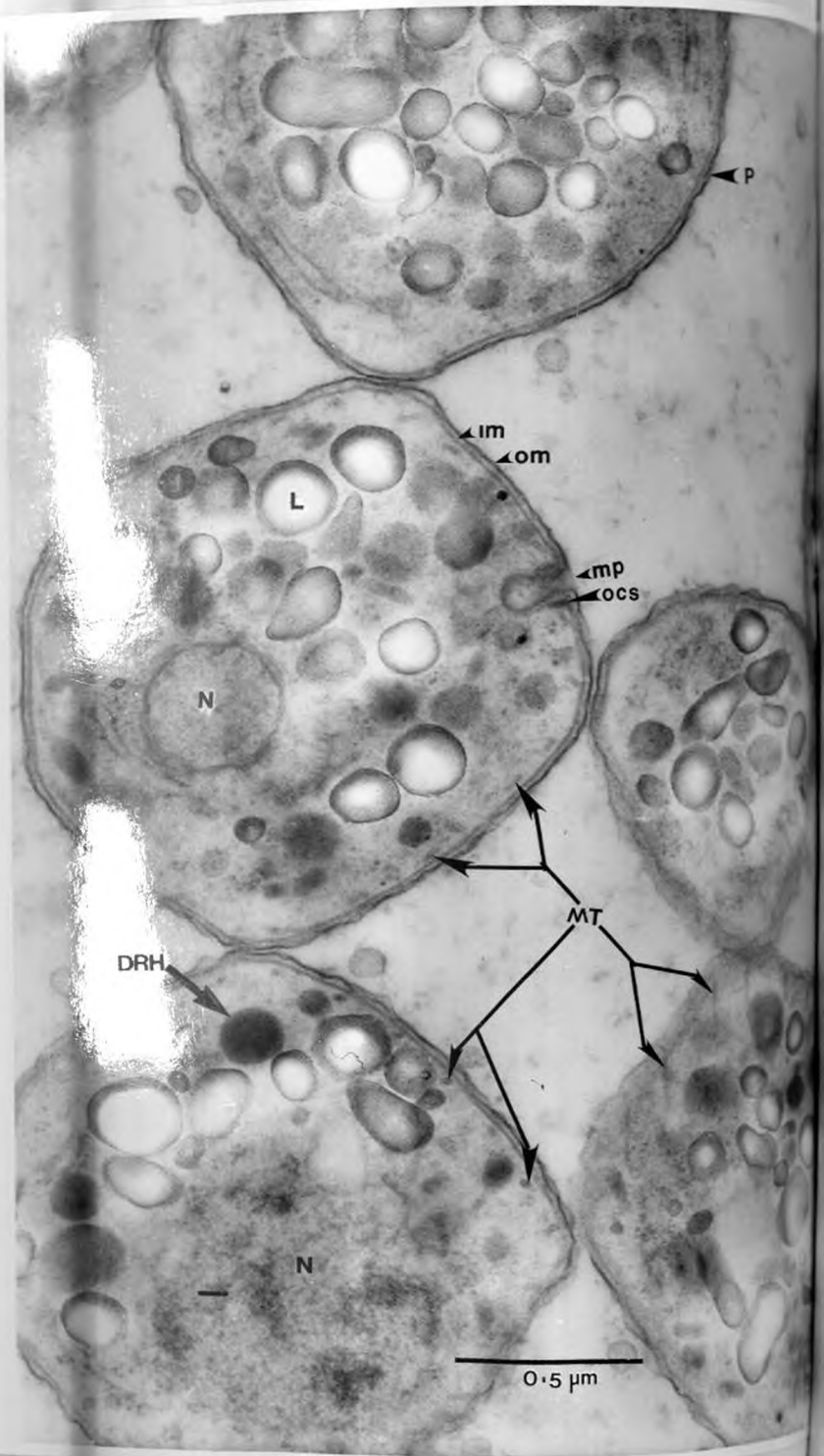

P
im
om
L
mp
ocs
N
MT
DRH
N
0·5 µm

Figure 47

An electron micrograph showing transverse and tangential sections of B. besnoiti cystozoites.

Note: The micropore (MP) formed by outer membrane (OM) and inner membrane (IM) of the pellicle (P), the outer cylindrical structure (ocs) is formed solely from the (im), the oblique nature of the microtubules (MT) the ducts of rhoptries (DRH), the lipid (L) and amylopectin (AP) granules. (Uranyl acetate x56000)

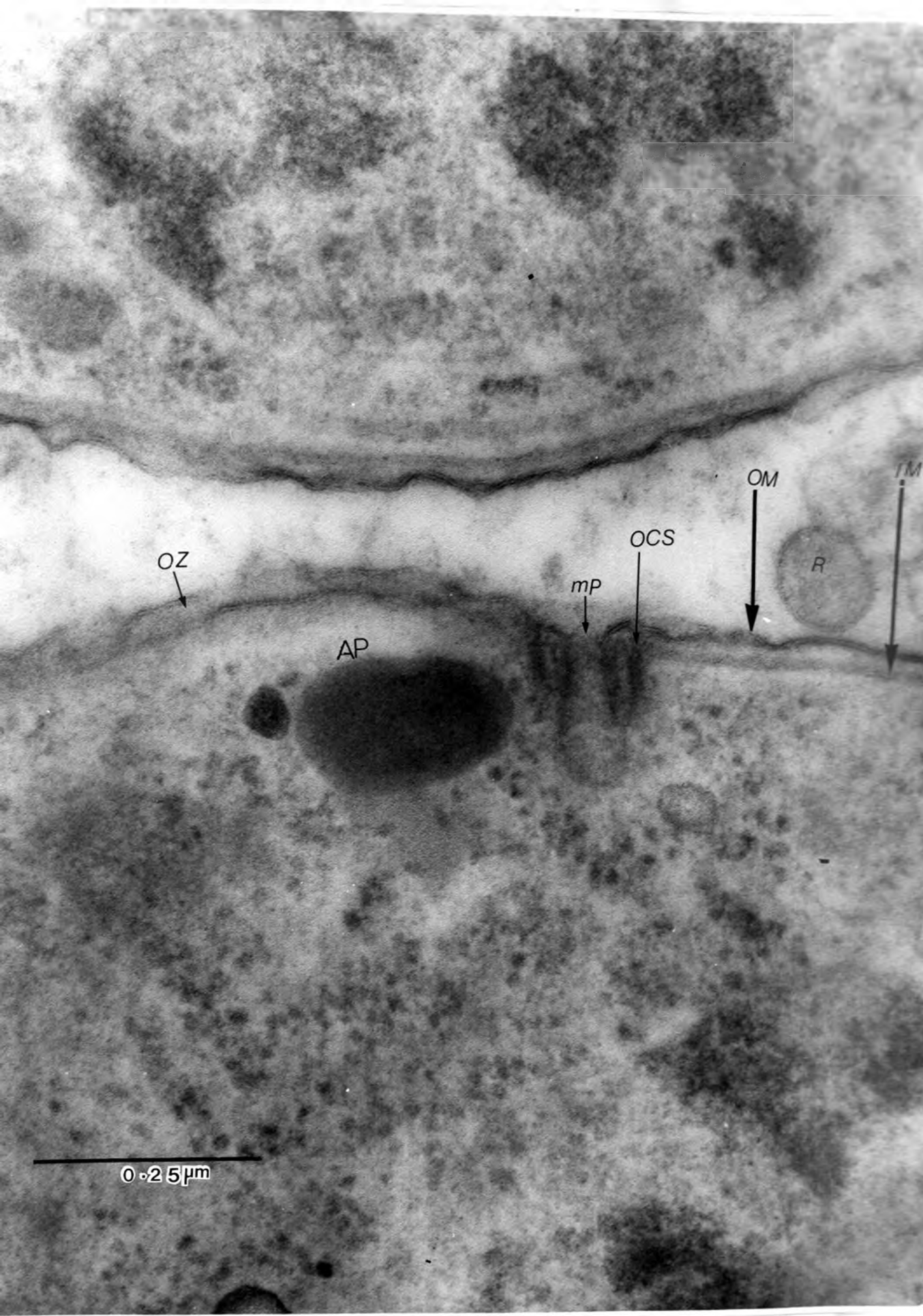

OZ
AP
mP
OCS
OM
R
TM
0·25μm

Figure 48

An electron micrograph showing the fine details of a longitudinal section of the micropore (MP) of Besnoitia besnoiti.

Note: The outer cylindrical structure (OCS) of the micropore appears to be principally formed from the inner membrane (IM) of the pellicle. The electron lucent zone (OZ) separating the outer (OM) and inner membrane of the pellicle is uneven. A ribosome (R) was found free in the cyst fluid while an amylopectin (AP) granule was observed in the cytoplasm. (x168000)

CHAPTER 5

5.0 DISCUSSION

The results of the survey indicate that both caprine and bovine besnoitiosis are important diseases in Kenya. Caprine besnoitiosis is endemic in a continuous belt extending from Coast Province through North Eastern, Eastern, and Northern Rift Valley. Nairobi and Kajiado are also affected. This confirms the assertion that the disease may be endemic in the Northern parts of Kenya and only a more detailed survey can give the true position (Bwangamoi, 1968).

The reason why Nairobi has such a high incidence (25.4%) compared to the neighbouring districts of Machakos (4.67%), Kajiado (1%) and Kiambu (0%) was due to the marketing of sick animals from North Eastern and Eastern provinces which were brought in lorries. This may also explain why no kids were infected as market goats were always adults that were only kept for a short time before slaughter.

Caprine besnoitiosis was not diagnosed in Central, Western and Nyanza provinces as well as in Central Rift Valley where farming is on small scale farms and goat rearing is a minor agricultural activity. Narok was surveyed intensely and though goats, sheep and cattle rearing are the main activities, no besnoitiosis was diagnosed.

This may call for re-location of the goat breeding stations from Eastern (Marimanti and Kiburine) and Coast

(Bachuma and Matuga) to areas where the disease does not occur. This is because the infected goats from the breeding stations could easily act as a means of disseminating the disease to the different parts of the country.

In some areas like Tana River and Baringo which neighbour districts where caprine besnoitiosis was confirmed, lack of diagnosis of the disease may be due to the low number of goats examined. This was because of inaccessibility of most parts. Thus there is need to continue with the search for the disease in this district.

Reports on caprine besnoitiosis in Kenya were 17 years apart (Bwangamoi, 1967 and Heydorn, et.al., 1984). The reason for this long hiatus is lack of awareness about the disease among qualified veterinary personnel. Indeed many expressed surprise that the disease was so easy to diagnose when shown how to do it using the method of checking cysts in the eye (Bigalke and Neude, 1962) during the survey. The meat inspectors who knew the disease when presented with a heavily parasitised carcass erroneously called it Sarcocystis. There is thus need to emphasise the disease during training of the veterinary staff at all national levels.

Bovine besnoitiosis occurs widely in the world. However the endemicity of the disease in Kenya was unknown (Bigalke, 1981). From this survey the disease was only found in Tana River district. This area borders the Tsavo East National Park and it is not clear what

role the wild animals may play in the epidemiology of this disease. There is need to examine more cattle in the neighbouring districts of Garissa, Kitui, Kilifi and Lamu in order to determine the true picture. However accessibility remains difficult.

No sheep was found with besnoitiosis through out the survey and this is in contrast to previous reports of finding three infected sheep (Bwangamoi et. al., 1989). However confirmation on histopathology was not done (Bwangamoi, 1989). It is possible that what was seen as conjuctival cysts were bulbo-tarsal glands which were easy to confuse with cysts. The fact that there is only one report on besnoitiosis in sheep (Hicks, 1982) makes it an unlikely host of Besnoitia.

The goat breeding stations had high infection rates: Marimanti 46.4%, Matuga 36% and Bachuma 34%. While the reason for this may not be quite clear, the crowding of the animals during the late evening, night and early morning was found in all the stations and may facilitate both direct or vector assisted transmission.

Bigalke (1967) found that chronically infected cattle transmitted the disease to non-infected cattle when allowed to cohabit but the mode of transmission was not clear, but he suspected biting insects. The presence of large number of ticks especially Rhipicephalus evertsi, Amblyomma variegatum and biting insects including Glossina species in these areas and the presence of large numbers of chronically infected goats may suggest that blood-sucking vectors play a role in the

transmission. The ability to reproduce the disease experimentally using intranasal inoculation, the presence of large numbers of cysts in the upper respiratory tract of the chronically sick goats, the presence of coughing in pneumonic goats and the overcrowding of the goats may also suggest that direct aero-transmission takes place. However these assertions need further detailed investigations.

According to Bwangamoi (1989) acute caprine besnoitiosis has not been observed. However during the survey and experimental transmission acute besnoitiosis was observed. The signs of fever, dullness, anorexia, lacrimation, oedema and pain in the testis are similar to those described for cattle by Bigalke (1981).

Infertility, abortion and neonatal deaths were reported in the goat flock of the epidemic previously reported by Bwangamoi, et. al.(1989). During this study infertility and abortions were observed confirming the observations of Bwangamoi et. al. (1989). From field and experimental observations infertility was caused by the bucks being unable to mount due to pain and aspermia associated with fever during the acute stage. In addition aspermia during the chronic stage was due to compromised blood flow to the testis by presence of large numbers of besnoitia cysts in the pampiniform plexuses. Chronically sick does were found to conceive and kid normally.

Abortions that were observed during this study in all cases occurred during the acute phase and only in

animals that had fever and generalised oedema. The presence of oedema in the placenta of the aborting does suggests that the oedema may cause abortion by anoxia as no other abnormality was observed in the aborted fetuses. However further research is necessary to confirm this assertion.

Alopecia and hyperkeratosis reported by Cheema and Toofanian (1979), Bwangamoi et. al. (1989) and Bwangamoi (1989) were also observed during this study. Except for the ears where alopecia was not associated with high numbers of cysts, all the other areas (face, carpus, hock and brisket) had a high number of cysts in the skin and subcutis.

It was observed that body condition and alopecia accompanied with hyperkeratosis were correlated to the number of cysts in the eyes. All the goats in poor body condition had alopecia and hyperkeratosis and more than 100 cysts per eye while in 33.3% of goats in fair body condition with alopecia and keratosis had an average of 53 cysts. Goats in good body condition had no alopecia or hyperkeratosis and had an average of 18.9 cysts per eye.

On gross examination and histopathology the alopecic areas of the skin were found to have large numbers of cysts in the skin and subcutis. The superficial blood vessels, intermuscular fascia of superficial muscles, teats, pampiniform plexus, tunica albuginea, tunica vaginalis and in upper respiratory tract of the same goats, were found large number of cysts. Similar

findings were reported by Cheema and Toofanian (1979), Bwangamoi et. al. (1989) and Bwangamoi (1989).

There was inconsistency in the cyst densities in the nasal cavity. Thus in some animals many cysts could be found in the trachea, turbinates and larynx while in others very few or no cysts were found. The reason for this is unclear and needs investigation.

Cheema and Toofanian (1979), Bwangamoi et.al. (1989) and Bwangamoi (1989) reported that deep seated organs in goats had fewer cysts while the superficial ones had many cysts. Basson et. al. (1970) found the same in cattle. During this study similar observations were made.

The large number of cysts in the epididymis, fibrosis of the testis, poor semen characteristics of chronically sick bucks and complete aspermia observed on histopathology in some bucks is consistent with the findings of Cheema and Toofanian (1979), Bwangamoi, et.al. (1989) and Bwangamoi (1989). It also confirms the assertion by other researchers that besnoitia organisms exhibit genitotropism leading to sterility in males in a variety of ruminants namely domestic cattle (Bigalke, 1967; Basson et. al. 1970; Nobel, et. al. 1981), Kudus, blue wildebeest (McCully, et. al. 1966), reindeer and caribou (Hadwen, 1922; Wobeser, 1976).

Histopathology confirmed the cyst distribution observed grossly. All the cysts had the basic besnoitia structure of a parasitophorous vacuole, surrounded by an inner cyst wall then the host cell, then a secondary cyst

wall and host animal connective tissue. Heydorn et.al. (1984) and Cheema and Toofanian (1979) reported similar findings in besnoitia of goats in Kenya and Iran respectively. A similar structure was reported for other besnoitiosis namely B. jellisoni (Ernest, Chobotar, Oaks and Hammond, 1968), B. besnoiti (McCully et. al. 1966; Basson et. al. 1970), B. wallacei (Frenkel, 1977) and B. tarandi (Hadwen, 1922).

The cyst diameters varied from 96µm to 781µm with the mean of the largest cysts being 461.6µm in natural chronic cases and from 14µm to 789.3µm in serially killed artificially infected goats killed from day 3 to day 305 post inoculation. The cyst wall thickness varied from 10-96µm with the mean of the largest cysts as 31.1µm. The size of besnoitia cysts varies in different species (Foley, Anderson and Steinberg, 1990). Thus B. besnoiti in cattle is on average 394µm, in wildebeest 567µm and impalas 472 µm (Bigalke, van Niekerk, Basson and McCully, 1967), in the epididymis of goats 132 x 185µm (Cheema and Toofanian, 1979), in reindeer 100-450µm (Hadwen, 1922), in goats in Kenya 150 x 310µm (Bwangamoi, 1989) and 0.5-1.5mm (Heydorn et al., 1984). This great variation of besnoitia cysts sizes reported by different researchers and observed in this study confirms the observation by Basson et.al. (1970) that cyst size is subject to age of the cyst. In addition the tissue involved appears to play a role in determining the size of the cysts. Thus when the means were taken for the largest cysts in blood vessels the cysts were in almost all cases 404µm in

diameter and a cyst wall of 19µm.

The pathogenesis of caprine besnoitiosis has not been previously described. However from the field and experimental serial studies conducted it is logical to conclude that the animals get infected with tachyzoites (cystozoites/endozoites) through nasal or parenterally by arthropod bites. This is followed by multiplication by endodyogeny in endothelial cells of blood vessels especially of the skin fascia and upper respiratory tract, producing more tachyzoites which invade other cells. Some of the released tachyzoites (endozoites) find their way into circulation in large numbers in some goats leading to acute besnoitiosis with fever, oedema, orchitis and abortions. During this phase cyst formation commences. The cystozoites multiply in activated histiocytic cells by endodyogeny and as this happens the host cell enlarges and becomes multinucleate. The cysts enlarge upto 789µ when mature approximately 112 days post-inoculation.

The large numbers of cysts in the dermis and fascia of the skin is responsible for the hyperkeratosis and acanthosis observed in the chronically sick goats, while their presence in the pampiniform plexuses is responsible for chronic aspermia observed in bucks. In these cases blood flow is compromised. These findings are similar to those reported by Pols, (1960), Bigalke, (1968) and Basson et. al. (1970). However the finding of young cysts at different stages of development in chronically sick goats held in isolation for over two years and

histopathological evidence of disintegrating cysts in the same goats indicates that the latter can give rise to further cysts in the same animal. This is in contrast to reports by Pols (1960), Bigalke (1968) and Basson et. al. (1970) who stated that new cysts do not form from disintegrating cysts in cattle infected with B. besnoiti.

According to Bigalke (1981) B. besnoiti cystozoites measured 8.4 x 1.9µm but ranged from 6.7-10.6 x 1.5-3.7µm. During this study the cystozoites of caprine besnoitia measured 8.5 x 2.4 and ranged from 6.0-10.3 x 1.9-3.1µm which makes these two Besnoitia species almost identical in their sizes.

Cheema and Toofanian (1979) reported that besnoitia cyst walls of goats stained faint blue or light red with haematoxylin and eosin (H. & E.), deep red with periodic acid schiff (PAS), dark blue with Masson's trichrome (MT) and light to bright pink red with Van Gieson's (VG) indicating its probable collagenous nature. However no attempt was made to indicate which stains were suitable for staining the different components of the besnoitia cyst.

During this study the best general stain was found to be haematoxylin and eosin. Giemsa, Gram, Van Gieson, PAS, Toluidine blue and Feulgen for demonstrating the cysts wall. Giemsa and PAS were good in demonstrating host cell nucleus, PAS, Toluidine blue and Feulgen were found good for demonstrating cystozoites. DNA/RNA pyronin were found good for demonstrating areas with wall forming body 1. It is worth doing further

research to try and relate the staining characteristics and the biochemistry of the organisms.

The initial experimental observation showed that the caprine besnoitia does not infect rabbits, hamsters, guinea pigs, rats, mice and cattle. Thus none of these animals can act as laboratory models for studying caprine besnoitia. Rabbits are more susceptible to Besnoitia besnoiti than the natural host - cattle and have been widely used in studies of bovine besnoitiosis (Pols, 1960; Bigalke, 1967; Shkap, Marcowitz, Pipano and Greenblath, 1982).

The inability of the caprine besnoitia to cause disease in cattle and rabbits indicates either a great difference in its pathogenicity in these animals compared to B. besnoiti or has a strong host specificity for goats. The latter seems more logical, as all the goats inoculated parenterally and intranasally got besnoitiosis, regardless of the sex, whether using blood containing bradyzoites or cystozoites derived from fascia as the infective material, while all the cattle and rabbits were refractory. Inoculation of cattle and rabbits with field isolates of B. besnoiti and development of clinical disease in both confirmed the differences in infectivity of these two besnoitias.

During an epidemic of besnoitiosis in goats reported by Bwangamoi et. al. (1989) none of the 36 cattle that had been grazing alongside the goats for 2 years had any cysts in their eyes. During the survey period to assess the extent of besnoitiosis in Kenya, it was found that

cattle reared together with flocks of goats with besnoitiosis were not affected. This was also true for goats when they were reared together with infected cattle as was found in Tana River. This calls for serious epidemiological studies as experimentally, goats have been found to be infected with B. besnoiti (Pols, 1960) and supported by the findings of this study that cattle previously exposed to caprine besnoitia were refractory to infection by B. besnoiti and both besnoitia species behave similarly when experimentally transmitted to their natural hosts.

Ultrastructural studies showed that both B. besnoiti and caprine besnoitia have typical apicomplexan features namely a three layered pellicle, conoid, rhoptries, microtubules, micronemes and polar ring. Similar features were reported for the related organisms Besnoiti jellisoni (Sheffield, 1966, 1968) and Toxoplasma gondii (Ogina and Yoneda, 1966; Sheffield and Melton, 1968; Aikawa et. al. 1977). These features have been reported for B. besnoiti (Neuman, 1974; Gobel, et. al., 1985; Shkap, et. al., 1988) and in a goat Besnoitia in Kenya (Heydorn et. al., 1984).

The smooth pellicle in caprine besnoitia compared with the wavy one of B. besnoiti explains the differences in width of the electron lucent zone separating the outer and inner membranes of the pellicle in the two species.

The micropore of B. besnoiti and caprine besnoitia were different in their structure. It therefore appeared that the two were different species as micropore

structural differences were species specific (Scholtyseck and Mehlhorn, 1970).

Twenty two regularly arranged microtubules (subpellicular fibrils) in the two species is the same number found in B. jellisoni (Sheffield, 1966) and in T. gondii (Sheffield and Melton, 1968). The microtubules in B. jellisoni extended posteriorly beyond the region of the nucleus and on tangential sections appeared curved indicating that they were spiral around the cell periphery (Sheffield, 1966). B. besnoiti resembled B. jellisoni in the spiral arrangement of its microtubules. However caprine besnoitia differed with B. jellisoni because the microtubules were not arranged in a spiral.

The micronemes were highly pleomorphic and randomly arranged in B. besnoiti and caprine besnoitia which indicated that they were highly convoluted. This agrees with previous reports on other sporozoans (Scholtyseck, 1970).

Wall forming bodies are features of the macrogametes of all Eimeria species studied, in which two large granules W1 and W2 fuse to form typical two-layered oocyst. The first type W1 are membrane bound homogenous and electron dense and usually have a peripheral location. The second type W2 are spongy-like fine structures mostly in the central region of the cytoplasm (Scholtyseck, 1970). In contrast in B. besnoiti the W1 is not homogenous but composed of a large membrane bound body with many small electron dense membrane bound bodies. In caprine besnoitia W1 is similar to that

described for Eimeria species. It was in all cases found in close proximity to the nucleus. In the two species the W2 were similar to that reported for Eimeria species (Scholtyseck, 1970).

The relationship of W1 and W2 in B. besnoiti is not clear and needs further studies. On the other hand W2 in caprine besnoitia appeared to be formed from W1. The W2 reported in this study resembles the unknown structure reported in B. jellisoni (Sheffield, 1966).

The reasons why the W1 and W2 have not been described in previous reports on B. jellisoni (Sheffield, 1966, 1968) and B. besnoiti (Neuman, 1974; Gobel, et. al. 1985; Shkap, et. al. 1988) are not clear. However most of these reports are based on cultured material, which may have caused the parasite to behave differently. Secondly, where cysts were obtained from natural host (Shkap, et. al. 1988) the cystozoites had been released from the cyst. During this study the W1 and W2 were only observed in some cysts and within those cysts, only in some cystozoites, so disruption could easily make one miss the wall forming bodies. The presence of two W2 bodies in cystozoites of the caprine besnoitia and a fusing or dividing W2 in B. besnoiti may indicate that they may have a role in replication. However only serial ultrastructural studies can clearly elucidate the role and development of W1 and W2.

The marked differences in the content of amylopectin and lipids in the cystozoites of the two besnoitia species may indicate differences in metabolism. This

needs further investigation.

The nucleus of caprine besnoitia was always surrounded by a halo zone while that of B. besnoiti was in most cases, but not always possible to discern the nuclear membrane. Processing the tissues may have contributed to the apparent loss of the nuclear membrane. But since both sets of tissues were processed as a batch and no trace of the nuclear membrane was seen in the caprine besnoitia, it is possible that it has a different lipid composition to B. besnoiti. The cause of the granular appearance of chromatin in B. besnoiti and the relative homogeneity in the caprine besnoitia is not clear. It is necessary to study the cytochemistry, physiology and biochemistry of these organisms. In addition serological studies should be done to find out the antigenic differences and how they relate to the ultrastructural features observed.

It has been proposed that ultrastructural features should be used as the chief criteria of distinguishing sporozoans (Honigberg et. al. 1964; Garnham, 1969; Levine, 1969a,b). In addition host and site specificity are important features in distinguishing sporozoans (Marquardt, 1970). During this study ultrastructural differences in the pellicle, micropore, microtubules, wall forming bodies, amylopectin, lipids and nucleus were observed. This in part explains why the two species B. besnoiti and the caprine besnoitia have a different host range.

It is therefore logical to conclude from this ultrastructural study and the differences in host range that the caprine besnoitia is a different species from B. besnoiti. The name Besnoitia caprae is suggested for the pathogen that causes besnoitiosis in goats.

CHAPTER 6

6.0 CONCLUSIONS

1. Caprine besnoitiosis is widely endemic in Kenya leading to serious negative economic losses due to abortions, sterility in bucks, poor weight gain, condemnation of meat and skins.
2. Bovine besnoitiosis is not widely distributed but where it occurs like in Tana River district it causes serious losses similar to those of goats.
3. Adult goats have higher infection rates than young ones.
4. There are no differences in infection rates between sexes.
5. Sheep are not naturally affected by besnoitiosis.
6. Besnoitiosis predisposes goats to pneumonia especially that caused by Pasteurella haemolytica.
7. Besnoitia cysts occur in large numbers in the skin, subcutis, upper respiratory tract, intermuscular fascia of superficial muscles and peripheral blood vessels. Deep seated organs are rarely affected.
8. caprine besnoitia cysts resemble those of other besnoitia species.
9. H. & E. is the best general stain for besnoitia cysts while Van Gieson, Feulgen, Periodic acid schiff, Toluidine blue, gram and Giemsa are good differential stains.
10. Caprine besnoitia is not experimentally infective to cattle, rabbits, rats, mice, hamsters and guinea pigs but is infective to goats.

11. Besnoitia infection in goats does not elicit significant haematological changes.
12. Besnoitia besnoiti is infective to cattle, rabbits and goats.
13. Exposure of animals to caprine besnoitia protects susceptible animals from Besnoitia besnoiti.
14. Experimental transmission of caprine besnoitia to goats was possible through subcutanous, intravenous, intradermal, intranasal, intraocular and implantation routes. It was not possible through intrauterine, ocularly and orally.
15. Abortions only occur during the anasarca stage of the disease.
16. Pathogenesis of caprine besnoitia is similar to that of cattle except that cystozoites from existing cysts break away to form other cysts.
17. Self cure occurs in caprine besnoitiosis.
18. Both caprine besnoitia and Besnoitia besnoiti are typical apicomplexa.
19. Caprine besnoitia vary with Besnoitia besnoiti in the nature of their pellicle, micropore, amylopectin and lipid composition, nucleus, wall forming body one (W1) and microtubules.
20. From the differences in host range and ultrastructure caprine besnoitia is a different species from Besnoitia besnoiti. The name Besnoitia caprae is suggested for the caprine species.

Proposals for Further Studies

1. Complete survey to cover the districts not visited.
2. Study of the role of ticks Rhipicephalus evertsi and Amblyomma variegatum in natural transmission.
3. Cell culturing of Besnoitia caprae and production of a vaccine.
4. Study of the genetics of goats to find whether the animals free from the disease in endemic areas are besnoitia resistant and thus select them for breeding.
5. Serial ultrastructural studies to determine the mode of reproduction of these organisms and the role of W1 and W2 in this process.

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