Pathology and Pathogenesis of TRYPANOSOMA VIVAX Infection in Cattle

By

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DECLARATIONS

I BENJAMIN KIPKEMOI arap KIMETO declare that this thesis is my original work and has not been presented for a degree in any other University.

Signed.....

CANDIDATE

Date 20-2-1990

I PROFESSOR GERALD M. MUGERA declare that this thesis has been submitted for examination with my approval as the University Supervisor.

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ABSTRACT

A study on the pathogenesis of haemorrhages, tissue damage, disseminated intravascular coagulation and thrombocytopenia in *Trypanosoma vivax* infected cattle was carried out in order to determine the clinico-pathological changes, ultrastructural changes in tissue, peripheral leukocytes and platelets, haematological parameters, levels of parasitaemia, thrombocyte counts, megakaryocyte changes, PPC, PFC and PST.

Ten animals were infected with *Trypanosoma vivax* (KETRI 2445) and four served as controls. The study was carried out over a period of 28 days. Two animals died 22 days post infection and the others were sacrificed in extremis.

Clinically, the infected animals had pyrexia, tachycardia, tachypnoea, rough hair coat, anaemia, haemorrhages, inappetence, salivation, incontinence, depression, nasal and lacrimal discharge, diarrhoea, meleana, emaciation, bilateral corneal opacity, incoordination of hind limbs and recumbency. The gross and histopathological lesions included haemorrhagic pancarditis, pulmonary oedema, interstitial and glomerular nephritis, haemorrhagic gastroenteritis, splenic and hepatic hemosiderosis and erythrophagocytosis, thrombosis, icterus and mononuclear cell infiltration in tissues.

Haematological findings revealed reduction in PCV, RBC, Hb, WBC and thrombocyte count. All the animals tested positive for PST. The PPC and PFC values were not significantly altered. Thrombocytopenia was inversely proportional to parasitaemia. Ultrastructural changes included increased activity of the leukocytes, platelet and microvascular damage and extravascular localization of trypanosomes.

It can therefore be concluded that the haemorrhages observed in the present study were due to thrombocytopenia, DIC and microvascular damage induced by *T. vivax*. Platelet destruction, platelet consumption as part of DIC and dysthrombopoiesis were the cause of thrombocytopenia which was inversely proportional to parasitaemia.Severe haemorrhagic pancarditis was one of the common lesions. The parasite was localized extravascularly confirming that *T. vivax* is both a plasma and a tissue parasite.

CHAPTER 1

1. Introduction

Trypanosomiasis is a disease of humans and many domestic animals caused by trypanosomes and transmitted mostly by blood-sucking insects. African human trypanosomiasis "Sleeping sickness" is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense.* In Central and South America human trypanosomiasis is caused by *Trypanosoma cruzi* and is called "Chagas" disease. The African animal trypanosomiasis affecting cattle is known as "Nagana" and is caused by three trypanosome species, namely, *Trypanosoma (Trypanozoon) brucei brucei, Trypanosoma (Nannomonas) congolense* and *Trypanosoma (Duttonella) vivax.* In pigs, the disease is caused by *Trypanosoma simiae. Trypanosoma equinum* causes "Mal de Caderas", an equine disease found in tropical and subtropical America. The disease caused by *Trypanosoma evansi* is called "Surra" and is found in Asia and North Africa and that caused by *Trypanosoma equiperdum* is called "Dourine" and is found in several European countries.

African trypanosomiasis is transmitted by tsetse flies, and when infected tsetse feeds on uninfected animal, metacyclic trypanosomes are inoculated into the skin where they develop and then move to the regional lymph nodes and into the blood stream where they multiply rapidly.*T brucei* and *T. vivax* may also invade

the tissue and in the later stages of infection, all the three trypanosome species may be observed in the central nervous system.

Trypanosoma vivax infection is widespread in Africa. It is also found in South America, Mauritius and outside the tsetse belt in Africa where it is transmitted mechanically by tabanid horse flies. The infection is characterized by fever, anaemia, progressive weakness and emaciation. In West Africa, trypanosomiasis is acute and therefore of economic significance. In East Africa, the disease is usually mild but acute outbreaks have been reported (Cornell, 1936; Lewis, 1948; Mwongela *et al.* 1981; Wellde *et al.*, 1983 and Olubayo and Mugera, 1987). In acute cases, the disease is characterized by fever, anaemia and haemorrhagic diathesis. The nature and pathogenesis of the haemorrhagic reactions and tissue damage induced by this haemorrhagic strain of *T. vivax* are poorly understood.

This project was designed to study the pathogenesis of haemorrhages, tissue damage and mechanism of thrombocytopenia in cattle experimentally infected with a haemorrhagic strain of *T. vivax* with the following objectives:-

1. To determine the ultrastructural changes induced by *T. vivax* infection in the platelets and peripheral leukocytes.
2. To determine the clinical signs, macroscopic, histopathological and electronmicroscopic changes in body tissues including blood vessels induced by *T. vivax* infection.

3. To determine haematological parameters, levels of parasitaemia, platelet counts, megakaryocyte changes, total plasma protein concentration, plasma fibrinogen concentration, fibrin monomers and fibrinogen degradation products in *T. vivax* infection.

CHAPTER 2

2. Literature Review.

2.1. *T. Vivax Infection*

2.1.1. Trypanosoma (Duttonella) vivax

Trypanosomes are haemoflagellate protozoan parasites belonging to the genus *Trypanosoma* of the family *Trypanosomatidae,* order *Kinetoplastida,* class *Zoomastigophora* and phylum *Protozoa* (Hoare, 1972). *Trypanosoma vivax* is a salivarian trypanosome species of the subgenus *Duttonella* and first described by Ziemann in 1905.

2.1.1.1. Morphology

Trypanosoma vivax is 20 um or more in length, lanceolate with rounded posterior end and oval in transverse section (Hoare, 1972). It is generally monomorphic but dimorphic forms exist such as club-shaped and slender forms (Chardome and Peel, 1967). The slender forms may be mistaken for intermediate forms of the subgenus *Trypanozoon*. *T. vivax* is covered by a pellicle. It has a nucleus, a large kinetoplast, mitochondrion, a free flagellum and a well developed undulating membrane. An electron dense surface coat measuring 12 to 15 nm thick covers the pellicle

(Rudzinska and Vickerman, 1968). The surface coat is composed of glycoproteins usually referred to as variable surface glycoproteins (VSGs) (Cross, 1975). Chemical analytic studies of *T. brucei* (Allsopp, 1973) reveals that it has a major and a minor component with sedimentation coefficients of 4.5S and 1S respectively. The major component has a molecular weight of 40,000 – 80,000 daltons. In *T. vivax*, the molecular weight is between 40,000 – 50,000 daltons.

2.1.1.2. Life cycle.

When a tsetse fly feeds on an infected animal, it ingests trypanosomes along with blood. The parasites undergo a cycle of development within the tsetse proboscis, passing through procyclic and epimastigote stages and finally ending up as metacyclic forms. When the infected tsetse fly next feeds, infective metacyclic forms are injected into the skin of a mammalian host along with tsetse fly saliva. Metacyclic trypanosomes develop in the skin, invade the local lymph vessels and move through the regional lymph nodes into the blood stream as trypomastigotes where they multiply rapidly.

2.1.1.3. Culture

Several attempts have been made to culture *T. vivax in vitro*. Trager (1959, 1975) reported insect cultures, containing proboscis and thoracic tissue. Hirumi *et al.* (1983, 1984) described a technique by which the whole life cycle of *T. vivax* can be reproduced. These authors employed fibroblast feeder layers and

matrix gel green A beads onto which the parasites attach. Cultures were initiated either by blood of an infected mammalian host or the proboscis of an infected tsetse fly. All vector stages of the parasite developed in these cultures culminating in the production of metacyclic forms which were infective for cattle. It was observed that cultures initiated by an infected tsetse fly proboscis took longer to develop to infective forms. Brun and Moloo (1982) have reported that West African stock of *T. vivax* is easier to culture than East African stock of *T. vivax* .

Up to now the culture system in use for raising the blood stream form of trypanosome *in vitro* requires a feeder layer of mammalian cells, either bovine fibroblasts or endothelial cells, and the regular addition of foetal bovine serum.

2.1.1.4. Identification.

T. vivax can be distinguished from the other two major salivarian trypanosome species, namely *T. congolense* and *T. b. brucei* by microscopic examination of tsetse fly organs and mammalian blood for the appearance of the parasite. *T. vivax* has a larger kinetoplast than *T. b. brucei* and a free flagellum as opposed to *T. congolense* (Hoare, 1972). The location of the parasite in the tsetse fly has also been used as a means of differentiation. *T. vivax* is located in the proboscis whereas *T. congolense* is located in the midgut, proventriculus and proboscis and *T. b. brucei* is located in the midgut, proventriculus and the salivary glands. Subinoculation into rodents is another method of differentiation. *T. vivax* is not

easily raised in rodents while *T. congolense* and *T. b. brucei* readily infect rodents.

Biochemical methods have also bee used for identification. Such methods include isoenzyme analysis (Murray, 1982; Allsopp and Newton, 1985), and DNA hybridization (Massamba and Williams, 1984). Up to now *T. vivax* from East and West Africa have been distinguished by the virulence of the infection; West African parasites causing more acute infection (Murray *et al*,. 1984; Gardiner and Wilson, 1987).However, DNA hybridization and isoenzyme analysis can be employed. Molecular karyotypring is a method that can also be used to differentiate trypanosome species.

2.1.2. Epizootiology.

Trypanosoma vivax occurs in most parts of West, Central and South Africa. According to FAO – WHO – OIE, 1983, tsetse flies infest approximately 10 million Km^2 of Africa, representing 37% of the content and affecting 37 countries. Currently 30% of the 147 million cattle in countries affected by tsetse flies are exposed to infection (Murray and Black, 1985). The host of *T. vivax* are ungulates including cattle, sheep, goats, horses and camels to which it is pathogenic. Wild bovidae do not suffer from clinical disease but are carriers and therefore reservoirs of the infection. *T. vivax* is non-pathogenic to pigs, dogs and cats and laboratory animals (Hornby, 1949; Soltys and Woo, 1977). Moloo (1982) and Anosa (1983) have described *T. vivax* infection in domestic animals and rodents, and

Davis (1982) has described it in experimental laboratory, domestic and wild animals.

T. vivax is transmitted by *Glossina* species, notably *G. morsitans, G. pallidipes. G. longipalpis, G. palpis and G. fuscipes* (Hoare, 1972). *T. vivax* is readily transmitted by mechanical inoculators, especially the horse flies (*Tabanidae*) and stable flies (*Stomoxys*). These insects have made the area of distribution of *T. vivax* extend beyond the tsetse belt in Africa. It is also found in Mauritius, Central and South America where it was probably imported in cattle at least 150 years ago (Murray and Black, 1985).

2.1.3. Pathogenesis.

Metacyclic forms inoculated into the skin of a mammalian host by *Glossina* spp. develop and multiply causing a localized cutaneous lesion (Chancre) which contains mononuclear cell infiltration (Emery and Moloo, 1981; Dwinger *et al.*, 1986). The trypanosomes move through the lymph vessels to the regional lymph nodes (Adams, 1936; Hornby, 1952), causing lymph adenomegaly (Büngener and Mehlitz, 1977; Anosa, 1983). The lymph node enlargement is due to follicular hyperplasia, congestion and oedema. The parasite moves to the blood stream and then to all the body organs.. In the blood stream, infection is characterized by waves of parastaemia as trypanosome populations multiply, then most of the parasites die, but the few that survive begin multiplying again. There is fever, anaemia and thrombocytopenia (Davis, 1982; Wellde *et al.*, 1983). *T. vivax* can invade and damage the body tissues (Hornby, 1949, 1953; Büngener and Mehlitz, 1977; Van den Ingh and

Neijs-Bakker, 1979; Mwongela *et al.*, 1981; Wellde *et al.*, 1983; Olubayo and Mugera, 1987). *T. vivax* can also be found in the central nervous system. In chronic trypanosomiasis (*T. gambiense*), trypanosomes pass by way of the lymphatics into the subarachnoid space of the brain tissue and localize in the intercellular spaces (Stevenson, 1922).

2.1.4. *Clinical signs*

2.1.4.1. Cattle

The incubation period in the bovine is 9 to 14 days in the case of virulent strains and 9 to 59 days in the case of mild ones (Hornby, 1953). The course of *T. vivax* infection in West Africa is acute and the infection is induced by the short forms of the parasite, whereas in East Africa the disease tends to be chronic and is usually caused by the long forms of the parasite. The acute form leads to death within a week. The body temperature is high (40 – 41°C) (Maxie *et al.*, 1979). The animals stand with their heads hanging low. They show inappetence, increased respiration rate and they may be dyspnoeic. The diarrhoea is watery and even haemorrhagic (Wellde *et al.*, 1983). Parasitaemia is cyclic and high (Losos and Ikede, 1972). There is anaemia (Hornby, 1949; Mwongela *et al.*, 1981) as seen by the paleness of the mucous membranes of the gums, conjunctiva and in the vulva of a female animal. The hair coat is rough and there is weight loss (Hornby, 1949; Maxie *et al.*, 1979). The superficial lymph nodes are swollen (Anosa, 1983) especially prescapular and

prifemoral lymph nodes. There is nasal and lacrimal discharge. Abortions and stillbirths are observed in the pregnant cows. In non-pregnant cows the animals fail to come to heat and milk production is reduced in lactating cows. *T. vivax* infection has been reported in Kenya as a haemorrhagic disease by Hudson (1944), Lewis (1948), Mwongela *et al.* (1981) Wellde *et al.* (1983)and Olubayo and Mugera (1985). Morbidity rate is high (60%) and so is the mortality rate (100%) in infected Zebu cattle in West Africa (Hoare, 1972; Losos and Ikede, 1972). In East Africa, the morbidity and mortality rates are low (Hornby, 1929). In South America, Hoare (1972) reported a mortality rate of 30% in acute form of *T. vivax* infection. Uzoigwe (1986) reported a mortality rate of 30% in calves with acute *T. vivax* infection, and he attributed this to the ability of the calves to control parasitaemia and therefore anaemia. The same observation was made by Wellde *et al.* (1983).

2.1.4.2. Goats.

The incubation period is 4 to 15 days (Hoare, 1972). The course of the disease can be acute, subacute or chronic depending on the strain used. The disease is characterized by anaemia, rough hair coat, weakness, body weight loss, lacrimation, corneal opacity (Van den Ingh *et al.*, 1976; Saror, 1980; Anosa, 1983; Murray *et al.*, 1984). The lymph nodes are enlarged (Masake, 1984; Anosa and Isoun, 1983). The infection can be mild (Hornby, 1949) and the mortality rate can be low (12%) (Bruce *et al.*, 1913), or high (Lewis, 1947). Generally, *T. vivax* causes a more severe disease in West Africa than in East Africa (Murray et al., 1984) and this is due to the difference in

the pathogenicity between the West and East African Stocks of *T. vivax* (Gardiner and Wilson, 1987).

2.1.4.3. Sheep.

The incubation period is 4 to 14 days (Hoare, 1972). The pathogenicity varies from low to high depending on the virulence of the strain and the presence or absence of intercurrent helminthiasis or nutritional diseases. Anaemia is reported to be the major clinical sign (Clarkson, 1968; Anosa and Isoun, 1980). Mortality and parasitaemia are varied (Lewis, 1947; Losos and Ikede, 1972). The mortality rate can be high (70%) (Hoare, 1972).

2.1.4.4. Horses.

There is high temperature (Hornby, 1952). There are urticarial swellings in the saddle area of the horse's back. There is anorexia, conjunctivitis, icterus, scrotal oedema, oedema of the legs, latissimus dorsi, superficial muscles of the thorax and abdomen, muscle tremor and incoordination.

2.1.4.5. Camels.

Hornby (1952) reported that camels are susceptible to *T. vivax* infection and it may be acute or chronic.

2.1.4.6. Dogs.

Dogs are not susceptible to either experimental or natural *T. vivax* infection, (Losos and Ikede, 1972)

2.1.4.7. Laboratory animals

Several attempts have been made to establish *T. vivax* infection in the rodents (Desowitz and Watson, 1951; Leeflang *et al.*, 1967; Moloo, 1981, 1982). The pathogenicity and infectivity of rodent adopted *T. vivax* has been reported (Taylor, 1968).

2.1.5. *Clinical pathology*

Clinical pathological changes in *T. vivax* infected cattle have been described by Van den Ingh *et al.* (1976), Wellde *et al.* (1983) and Olubayo and Mugera (1987). The acute form of the disease is characterized by fluctuating parasitaemia, a decrease in total red blood cells, packed cell volume (PCV), haemoglobin (Hb), serum lipids and thrombocytes. There is an increase in blood urea and serum lactate dehydrogenase (LDH), a rise in d-amino laevulinic acid dehydratase (ALAD) and an increase in blood serotonin levels (Van den Ingh *et al.*, 1976). The decrease in albumin and gamma

globulin fractions during the first 5 weeks may be as a result of extravasation of serum caused by the action of vasoactive substances, increased protein breakdown and loss by protein urea or haemodilution. Decreased synthesis of albumin by the liver is unlikely as serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT) and sorbid dehydrogenase, indicators of liver damage are within normal range in *T. vivax infection* (Van den Ingh *et al.*, 1976). The increase of gamma globulin after the 5th week of infection may reflect a strong antibody response. Decreased serum lipids is due to increased need for glucose, lactic acid, pyruvic acid due to trypanosome metabolism, and glutamic acid disappearance from serum is due to an uptake by the trypanosomes. It has reported that it is an energy source. (Trager, 1974).

2.1.6. *Coagulation studies.*

Thrombocytopenia and disseminated intravascular coagulation (DIC) have been reported in human trypanosomiasis (Davis *et al.*, 1974; Robins – Browne *et al.*, 1975) and in experimental animal trypanosomiasis (Veenendaal *et al..*, 1976; Davis, 1982; Wellde *et al.*, 1978, 1983; Olubayo and Mugera, 1985; Ismail, 1988). However the mechanism of thrombocytopenia is not well described.

Thrombocytopenia can be caused by consumption of platelets as part of DIC (Barret-Connor *et al.*, 1973; Sadun *et al.*, 1973), immune platelet damage (Davis *et al.*, 1974) and splenic pooling in addition to immune platelet damage and platelet consumption as part of DIC (Robins - Browne *et al.*, 1975). When platelets are

damaged, they release a phospholipid factor (platelet factor 3) which initiates intravascular clotting. According to Thomson (1978), DIC may be caused by antigen-antibody complexes, tissue damage, intravascular haemolysis, endotoxin and hypersensitivity or by extensive endothelial damage. As fibrinogen, fibrin and factor V (Proaccelerin, labile factor) and VIII (anti-haemophilic factor) are all broken down during DIC, there is decreased chance for further clotting leading to haemorrhage.

Thrombocytopenia has also been reported in laboratory animals. It has been reported in rats experimentally infected with *T. rhodesiense* (Davis *et al.*, 1974) and *T. gambiense* (Greenwood and Whittle, 1976), and in Rhesus monkeys infected with *T. rhodesiense* (Sadun *et al.*, 1973).

Thrombocytopenia has been reported in goats (Veenandaal *et al.*, 1976) and cattle (Maxie *et al.*, 1983; Olubayo and Mugera, 1985 and Ismail, 1988) experimentally infected with *T. vivax*. DIC with haemorrhages has been reported in cattle (Losos and Ikede, 1972; Van den Ingh *et al.*, 1976). Thrombocytopenia always appears at the peak of parasitaemia (Davis, 1982).

In haemorrhagic diathesis described by Wellde *et al.* (1983) and Olubayo and Mugera (1985) in cattle infected with *T. vivax*, the authors reported thrombocytopenia, haemorrhages, elevated fibrinogen levels, extended prothrombin times and positive plasma paracoagulation test for presence of fibrin monomers and fibrinogen degradation products.

2.1.7. *Post mortem findings*

Macroscopic lesions in natural and experimental *T vivax* infection in animals has been reported by Hudson (1944), Hornby (1949), Fiennes (1954), Hoare (1972), Ikede and Losos (1972) Van den Ingh *et al.* (1976), Anosa and Isoun (1983) and Olubayo and Mugera (1987). In the acute form of *T vivax* infection in cattle, there are extensive petechial and ecchymotic haemorrhages (Hudson, 1944; Mwongela *et al.*, 1981; Wellde *et al.*, 1983 and Olubayo and Mugera, 1987) in the mucosal surface of the buccal cavity, larynx, pharynx, abomasum, small and large intestines and in the serosal surfaces of the peritoneum and the pleura, in the skeletal muscles, epicardium, endocardium, lymph nodes and adipose tissues. There is enlargement of the kidneys, the spleen and the liver (Fiennes, 1953), including anaemia and emaciation.

2.1.8. *Histopathology*

Several authors have reported histopathological changes in animals with *T vivax* infection (Lester, 1932, 1933; Hornby, 1949; Fiennes, 1953; Losos and Ikede, 1972; Büngener and Mehlitz, 1977; Van den Ingh and Neijs-Bakker, 1979; Maxie *et al.*, 1979; Anosa and Isoun 1983 and Olubayo and Mugera, 1987).

While working with *T. vivax*, Lester (1932, 1933) described changes in the myocardium. These changes include fatty degeneration, fragmentation and necrosis of fibres, haemorrhages, inflammatory cell infiltration and fibrosis. The author did not

observe extravascular localization of trypanosomes. Losos and Ikede (1972) carried out routine histological examinations in cattle infected with *T. vivax* for 1 to 3 months and found neither myocardial lesions nor extravascular localization of the parasite. This view was supported by Maxie *et al.* (1979). Other authors (Büngener and Mehlitz, 1977; Van den Ingh and Neijs-Bakker, 1979) have described mononuclear pancarditis with extravascular localization of trypanosomes. Büngener and Mehlitz (1977) and Masake (1980) have described histological changes in the lymph node and the spleen.

2.1.9. Host response.

The host response to *T. vivax* infection is increased production of IgM (Luckins, 1974; Clarkson *et al.*, 1975) which is associated with antibody production to successive variants (Van Meirvenne and Vervoort, 1977). *T. vivax* produces new variants every 2 to 4 days (Jones and Clarkson, 1974) and these appear in a clear order (Clarkson and Awan 1969) showing that they are genetically controlled. The variants are localized in the trypanosome variable surface glycoproteins (Cross, 1975). *T. vivax* antigens have been isolated (Lanham and Godfrey, 1970) and characterized (Murray, 1982; Massamba and Williams, 1984; Allsopp and Newton, 1985). According to Black *et al.* (1982), variable antigens are released by degenerating trypanosomes. There is increased production of IgM because it is more protective than IgG (Takayanagi and Enriquez, 1973).

The presence of large amounts of antigen-antibody complexes causes anaemia (Losos and Ikede, 1972) which is

haemolytic (Jennings *et al.*, 1974; Jennings, 1976; Mackenzie and Cruickshank, 1972; Woodruff *et al.*, 1973). Antigen-antibody reaction with complement leads to the release of histamines (Richards, 1965), serotonin and kinins (Boreham, 1970; Veenendaal *et al.*, 1976). These substances increase vascular permeability resulting in oedema and inflammation (Boreham, 1979). Glomerular injury is also associated with IgM and C_3 (Nagle et al., 1974). Antigen-antibody complexes also cause platelet aggregation, formation of microthrombi, and thrombocytopenia leading to haemorrhage (Barret-Connor *et al.*, 1973).

Tizard *et al.* (1978) and Boreham (1979) have reported that biologically active substances derived from the trypanosome could also account for major pathological changes – erythrolysis caused by haemolysins and phospholipases leads to anaemia; microvascular damage is caused by phospholipases, proteases and free fatty acids; immunosuppression by free fatty acids; and hypocomplementaemia by lipoplysaccharides and proteases.

Immunosuppression has been observed in host animals. The animals usually succumb to secondary infections such as pneumonia (Hoare, 1972). Holmes *et al.* (1974), while using a commercial polyvalent clostridial vaccine, observed reduction in antibody production. Urquhart *et al.* (1973) showed that *T. brucei* infected rats were unable to mount a normal response to the nematode *Nippostrongylus brasiliensis* and that the worms did not undergo immune expulsion. Pearson *et al* (1978) also reported that mice with trypanosomiasis showed delayed skin graft rejection. The cause of immunosuppression can be due to B cell defect caused by

mitogens produced by trypanosomes (Greenwood and Oduloju, 1978). It could also be due to T-B cell interaction and macrophage handling of the antigen (Urquhart, 1980) and free fatty acids (Tizard *et al.*, 1978). However, Clarkson (1976) believes that immunosuppression is linked with excessive production of IgM.

2.1.10. Host resistance.

In African trypanosomiasis, both acquired and innate resistance occur in cattle (Murray and Black, 1985).

The acquired resistance raises the possibility of vaccine production against the tsetse transmitted metacyclic trypanosomes which have smaller repertoire of variable antigens than the blood stream parasites. Vaccination can be attempted by infection and treatment (Wilson *et al.*, 1975), by using irradiated parasites (Wellde *et al.*, 1973), and by using immunization with metacyclics produced *in vitro*, killed and broken by ultrasonication.

Innate resistance is observed in trypanotolerant breeds of cattle. These animals develop smaller chancres in the skin following deposition of metacyclics by tsetse flies (Dwinger *et al.*, 1986). They also, have the capacity to control trypanosome growth and develop effective immune response and resist anaemia. They can also utilize food efficiently, conserve water , and resist heat (Murray *et al.*, 1982).

Breeds like N'Dama and Muturu (*Bos taurus*) can be maintained in trypanosomiasis endemic areas but not the Zebu (*Bos indicus*) (Chandler, 1952; Murray *et al.*, 1984). Trypanotolerance has a genetic basis which is perhaps related to haemoglobin types (Bangham and Blumberg, 1958) and rapid acquired immune

response. The less severe anaemia in N'Dama cattle is reflected in their capacity to control parasitaemia (Dargie, 1980) and therefore anaemia.

2.1.11. Control

The control measures which have to date been instituted to combat African animal trypanosomiasis have been targeted at the vector and the pathogen. There is also the possibility of using trypanotolerant breeds of livestock in tsetse infested areas as another method of trypanosomiasis control.

Several methods have been employed to control the tsetse fly, namely; (1). deprivation of tsetse habitat by clearing the bush mechanically or chemically, and by elimination of wild game which act as source of food (Ford *et al.*, 1970); (2). catching tsetse flies by hand (reviewed by Glasgow and Potts, 1970), by using biconical traps (reviewed by Allsopp *et al.*, 1985) which can be impregnated with insecticides, and by using screens which are impregnated with insecticides and baited with attractants (Vale and Hall, 1985, reviewed by Jordan, 1986); (3). ground and aerial spray with insecticides (Allsopp *et al.*, 1985); (4). biological control measures which entail the use of predators or pathogens (bacteria, fungi, protozoa) of tsetse flies (Nash, 1970; reviewed by Jordan, 1986) (5). genetic control method which is affected through the use of sterile males. This method involves induction of dominant mutations in the male gametes rendering them sterile (Dame, 1970) without reducing their inseminating capability.

The parasite control methods include vaccination, chemotherapy and chemoprophylaxis. To-date, there is no effective vaccination method because the parasite possesses a sophisticated mechanism of evading the host immune destruction. There are, however, two types of antigens, i.e. the antigens in the procyclics and the variable surface glycoproteins in the metacyclics, which are possible candidates for use in vaccination (Barry, 1986 and Nantulya, 1986). But there are several problems to immunization under field conditions using metacyclic antigens such as heterogeniety of metacyclic VATs for each serodeme, the number of serodemes present in each trypanosome species and the instability of VATs.

Several trypanocidal drugs have been used against *T. vivax* and *T. congolense* infection in cattle (Leach and Roberts, 1981; Meshnick, 1982), and these are:- (1) the phenanthridine group which include homidium bromide (Ethidium-Boots) or homidium chloride (Novidium-May & Baker), (2) the diamidine group which includes diaminazene aceturate (Berenil-Hoechst), (3) the phenanthridine-pyramidine group which includes pyrithidium (Prothidium-Boots). Animals that are exposed to infection and treatment regime are not only cured of the infection, but also develop a substantial degree of acquired immunity (Wilson *et al.*, 1976). The use of chemotherapy poses several problems such as drug resistance. Drug resistance is exhibited by various trypanosome species due to overdosing, irregular dosing and indiscriminate use of drugs without regard to trypanosome species present in the locality. Another problem is the development of cross-resistance by trypanosomes to many drugs (Leach and Roberts,1981).

The use of trypanotolerant breeds of domestic livestock can also be used as a control measure against trypanosomiasis. The animals can live and be productive in the tsetse infested areas of Africa thus increasing animal protein (FAO/WHO/OIE, 1983; Jordan, 1986) and be used as draught oxen for agriculture (Finelle, 1980).

CHAPTER 3

3. Experimental Infection of Cattle

3.1. Materials and Methods

3.1.1. Cattle

14 cattle were used – 6 Ayrshire steers and 8 Friesian bull calves. They ranged in the age from 4 to 24 months. They were fed on hay, ranch cubes, bran, salt and given water *ad libitum*. The animals were housed in a fly proof barn with 2 in each pen.

3.1.2. Pre-infection screening

The animals were screened for patent trypanosome infection by the Standard Trypanosome Detection Method (Wilson, 1969) before commencement of the experiments. Thick blood smears were prepared, from each animal, stained and examined as described by Baker (1970). Wet blood films were made by placing a drop of blood on a slide and covering it with a 22 x 22 mm cover slip; the entire preparation was then scanned using light microscope with x 10 eye piece and x 25 objective. Buffy coat was prepared as described by Woo (1970). The buffy coat zone was examined directly by a light microscope with x 10 eye piece and x 25 objective. The last screening method was mice inoculation using bovine blood. 0.5 ml of blood

from an experimental animal was inoculated intraperitoneally into 2 mice. Wet preparations of mouse tail blood were examined 3 times per week for 3 weeks before the mice were declared not infected. All the screened experimental animals were found to be negative.

3.1.3. *Trypanosoma vivax.*

T. vivax stock (KETRI 2445) was obtained from the Kenya Trypanosomiasis Research Institute (KETRI).

3.1.4. *Blood samples.*

Blood was collected in EDTA from the jugular vein three times per week, and used for haemotological studies, coagulation studies, parasitaemia determination, blood smear, thrombocyte counts, platelet and leukocyte separation.

3.1.5. *Parasitaemia estimation*

Estimation of parasitaemia was determined by the haematocrit centrifuge technique (Woo, 1970). The buffy coat was placed on the slide, covered with a 22 x 22 mm cover slip and examined under light microscope with x 10 eye piece and x 25 objective. The scoring was 2 + for presence of 1 – 10 parasites per whole preparation of cover slip and 4 + for the presence of 1 – 10 parasites per microscopic field as shown below:-

Scoring	No. of parasites	Parasitaemia
1 +	1 parasite per cover slip	10^2
2 +	1 – 10 parasites/cover slip	$10^2 - x\ 10^3$

3 +	10 parasites per cover slip	5×10^3
4 +	1 – 10 parasites per field	$10^4 - 10^5$
5 +	10 parasites per field	$5 \times 10^5 - 10^6$
6 +	100 parasites per field (Swarming)	5×10^6

3.1.6. Haematology

Blood samples were collected in bijou bottles containing EDTA.

3.1.6.1. Blood cell counts

The red blood cell counts and the white blood cell counts were determined using an electron particle counter (Coulter Counter Model SM, Coulter electronics Ltd. Northwell Drive Luton Beds LU3 3 RH England). The white blood cells were diluted 1:500. 20 ul of blood sample was mixed with 10 ml of buffered isotonic saline (Isoton), 6 drops of ZAP-Globin (a lysing reagent – Coulter electronics, Nairobi, Kenya) were added to lyse the red blood cells. A dilution of 1:50,000 was made for the red blood cell count. 100 ul of the sample used for white blood cells count was mixed with 10 ml of isoton.

The haemoglobin concentration (Hb) was determined by a haemoglobinometer attached to the Coulter Counter. The sample was prepared as described for white blood cell count. ZAP-Globin contains a cyanide reagent which reacts with haemoglobin to form a stable cyanmethaemoglobin which is measured by the haemoglobinometer.

The packed red cell volume (PCV) was determined by haematocrit centrifuge (Haemofuge, Heraeus Christ Gmbh Osterode, W. Germany).

3.1.6.2. Thrombocyte counts.

Blood was diluted 1:20 with 1% ammonium oxalate solution in a test tube and gently shaken. Both chambers of haemocytometer were filled with the dilution and incubated in a humid petri dish for 20 minutes in order to allow the platelets to settle. The haemocytometer was then placed under a light microscope and focused with x 10 eye piece and x 25 objective. The light was dimmed to make the platelets visible and while focusing up and down with the fine adjustment, all the platelets in five medium squares, that is, 5 x 16 = 80 small squares were counted. The number of platelets counted was multiplied by 1,000 in order to obtain the total number of platelets per cubic millimeter.

$$\text{Total no. of platelets/mm}^3 = \frac{\text{No of platelets counted (NPC)}}{\text{Surface area x depth x dilution}}$$

$$= \frac{\text{NPC}}{80 \times \frac{1}{400} \times \frac{1}{10} \times \frac{1}{20}}$$

$$= \frac{\text{NPC}}{\frac{1}{5} \times \frac{1}{10} \times \frac{1}{20}}$$

$$= \text{NPC} \times 1{,}000$$

3.1.6.3. Platelet separation.

Platelet separation was done according to the method described by Doeryl *et al.* (1970). 9 ml of blood was drawn into a disposable plastic syringe and rapidly put in a test tube containing 0.2 g EDTA. The stoppered test tube was inverted several times (x 10) in order to ensure sufficient mixing. The blood was then centrifuged at 300 x g for 15 minutes to obtain platelet rich plasma (PRP). The supernatant fluid was removed and the pellet was washed three times in cold EDTA – Tyrode solution and suspended in Krebs-Ringer bicarbonate buffer (pH 7.4). The suspension was centrifuged at 200 x g for 10 minutes. The pellet was then fixed as described by Karnovsky (1965) and processed for electron microscopy.

3.1.6.4. Peripheral leukocyte separation.

Blood sample was collected as described above for platelet separation. After the removal of the PRP, the buffy coat just below it was removed. It was then centrifuged at 1250 x g for 15 minutes and the pellet fixed as described by Karnovsky (1965) and processed for electron microscopy.

3.1.6.5. Total plasma protein concentration (PPC).

The PPC was determined by the method described by Schalm *et al.* (1975) using the Goldberg refractometer (American Optical Company, buffalo, New York).

3.1..6.6. Plasma fibrinogen concentration (PPC).

The PFC was determined by the method described by Schalm *et al.* (1975) employing the capillary tube.

3.1.6.7. Protamine sulphate paracoagulation test (PST).

PST is used to detect fibrin monomers (FM) in plasma following clotting. Fibrin monomers and fibrinogen degradation products (FDPs) are normally present in complex form in plasma. Polymerization of FM in plasma is therefore prevented by the presence of FDPs which hold FM in soluble complex. The addition of protamine sulphate to plasma containing FM/FDPs complex will release FM molecules from the complex and allow FM to polymerize to form visible insoluble fibrin.

PST was carried out using the method described by Seaman (1970). To 1.0 ml of plasma in a test tube (temp. 37°C), 100 µl of 1% protamine sulphate (Serva Feinbiochemica Heidelberg, New York) solution was added, mixed gently and the result read immediately and graded according to the amount of precipitate and the time taken for precipitation to take place.

5 + Fibrin clot or obvious flocculent precipitate formed soon after adding protamine sulphate.

4 + Fibrin clot or obvious flocculent precipitate formed within 20 seconds on the addition of protamine sulphate.

3 + Fibrin strands or obvious flocculent precipitate (usually less in consistency than for 4 + or 5 +) formed within 2 minutes of adding protamine sulphate.

2 + Small amounts of flocculent precipitate (smaller than for 3 +) formed 2 minutes after the addition of protamine sulphate.

1 + Flocculent precipitate present 20 minutes after adding protamine sulphate.

0 (Negative). No visible precipitate 20 minutes after adding protamine sulphate.

3.1.7. Bone marrow biopsy.

Biopsy material was obtained from sternum manubri between the 5th and the 6th intercostal space. The animals were restrained and the hair around the puncture area was shaved, washed and disinfected with 70% ethanol. 14 gauge needles and 10 ml disposable plastic syringes were used. The needle was pushed through the skin, the periosteum and into the marrow, and with a strong pull, the biopsy material was aspirated and placed on a clean slide. Films were then made, air dried and stained with Wright's stain and Giemsa stain. The myeloid, erythroid and megakaryocyte

counts were determined according to the method described by Schalm *et al.* (1975).

3.1.8. Clinical signs.

Temperature, pulse and respiration rates were recorded daily and in addition other clinical signs including inappetence, salivation, lacrimation, anaemia, depression, dyspnoea, corneal opacity, weakness, incontinence, diarrhoea, haemorrhage, incoordination, emaciation and recumbency were observed and recorded.

3.1.9. Post mortem examination.

Post mortem was performed on all experimental animals including the controls. Gross lesions were described and tissues from the lungs, lymph nodes, heart, liver, abomasum, intestines, kidneys, adrenals, skeletal muscle, brain and spinal cord were removed for histopathological examination. Tissues from organs showing diffuse haemorrhages (heart, spleen) were processed for electron microscopy, where special attention was paid to vascular lesions in these organs.

3.1.10. Histopathology

Tissues removed during post mortem examination and taken for histopathological examination were fixed in 10% formalin, and after embedding in paraffin wax, sections 5 microns thick were

cut and stained with haematoxylin eosin (H.E.) and Masson's trichrome stain and examined under a light microscope.

3.1.11. Electron microscopy

Tissues from the heart and spleen and platelet and leukocyte pellets were fixed as described by Karnovsky (1965) in formaldehyde-glutaraldehyde mixture containing 0.2% trinitrocresol for 2 hours at 4°C, washed three times (5 minutes each time) with 0.2 M phosphate buffer (pH 7.2) and post fixed with 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.2) for 3 to 5 hours. Blocks were washed 3 times (5 minutes each time) in isotonic saline solution, dehydrated in series of acetone,and embedded in Durcupan (Fluka, Basel, Switzerland).

Thin sections of 50 to 60 nm thick were cut using OM V-2 Reichert Ultramicrotome (Vienna, Austria), mounted on polyvinyl formal resin-coated copper grids and stained with uranyl acetate for 8 minutes and lead citrate for 3 minutes. They were then examined with a Zeiss EM 9 Electron Microscope (Oberkochen, W. Germany) using an objective diaphragm of 50 nm and photographs taken using Agfa Gevaert Scientia film (Belgium).

3.2. Experimental Designs and Procedures

3.2.1. *Experiment 1*

Clinical observations and pathological changes in *T. vivax* infected steers.

3.2.1.1. Introduction

In this experiment a haemorrhagic strain of *T. vivax* (KETRI 2445) was inoculated into steers in order to observe the clinical signs and pathological changes. The experiment was terminated after 28 days.

3.2.1.2. Experimental design.

Six Ayrshire steers aged between 18 and 24 months were used. Four steers (K 121, K 122, K 123 and K 124) were each infected with 5×10^5 trypanosomes/ml intravenously. Two (K 125, K 126) served as controls. 5 ml of blood in EDTA was obtained in a test tube from a donor steer experimentally infected with *T. vivax* (KETRI 2445) and diluted with phosphate saline glucose (PSG) pH 8.0 and adjusted to 5×10^5 parasites/ml. 28 days after the infection, the experiment was terminated.

3.2.1.3. Experimental procedure.

Clinical signs including temperature, determination of parasitaemia, pathological changes, electron microscopical examination of tissues, platelets and leukocytes were all carried out as described above.

3.2.2. *Experiment II*

Haematological observations in *T. vivax* infected bull calves.

3.2.2.1. Introduction.

From the results obtained in experiment I such as haemorrhage and anaemia, it was felt necessary to design another experiment to determine haemotological parameters including thrombocyte counts.

3.2.2.2. Experimental design.

Four Friesian bull calves ranging in age between 4 and 5 months were used. Three (K 128, K 129 and K 130) were each infected with 10^6 trypanosomes/ml intravenously and one (K 131) served as control. A donor calf was intravenously inoculated with stabilated stock of *T. vivax* (KETRI 2445) and when parasitaemia was high (10^7 trypanosomes/ml), the infected blood was diluted with PSG (pH 8.0)

and adjusted to 10^6 trypanosomes/ml. The experiment was terminated after 28 days.

3.2.2.3. Experimental procedure.

Blood samples were collected in bijou bottles containing EDTA three times per week. All haematological parameters including PCV, Hb, parasitaemia, thrombocyte counts, PPC, PFC and PST were determined as described above. Temperature, respiration and pulse rates were also determined, and clinical signs and pathological changes described.

3.2.3. *Experiment III*

Bone marrow studies in *T. vivax* infected bull calves.

3.2.3.1. Introduction.

After observing thrombocytopenia, leukopenia and anaemia in experiment II, it became necessary to study the changes in this haemopoietic organ to determine the myeloid: erythroid ratio and observe the megakaryocyte numbers and changes.

3.2.3.2. Experimental design.

Four Friesian bull calves aged 4 months were used, 3 (K 132, K 133, K134) were infected as described in the previous experiment. One (K 135) served as control. The experiment was terminated after twenty eight days.

3.2.3.3. Experimental procedure.

Temperature, respiration and pulse rates were recorded daily. In addition, clinical signs and pathological changes were described. Haematological parameters including PCV, Hb, and parasitaemia, thrombocyte count, PPC, PFC, PST, were all determined. Bone marrow biopsy as described above was performed on the 9th, 16th and 22nd days after infection.

CHAPTER 4

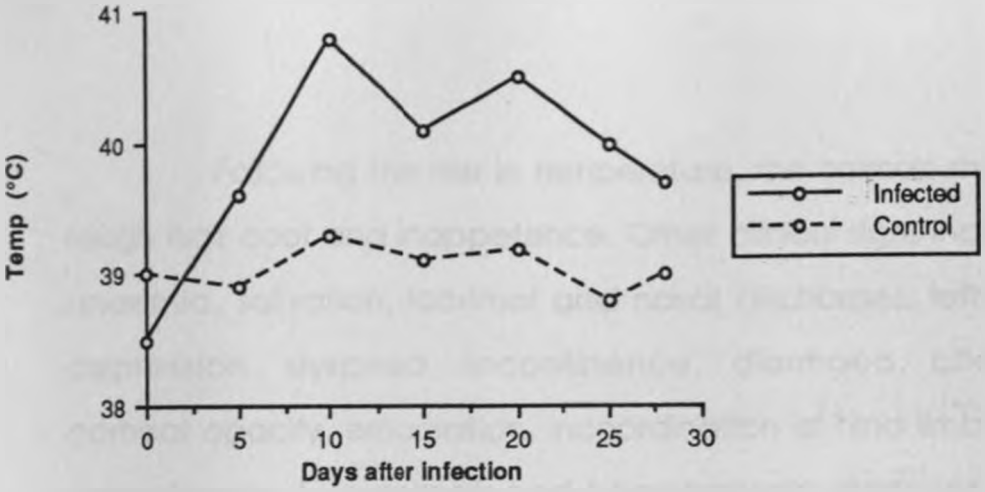
4. Results

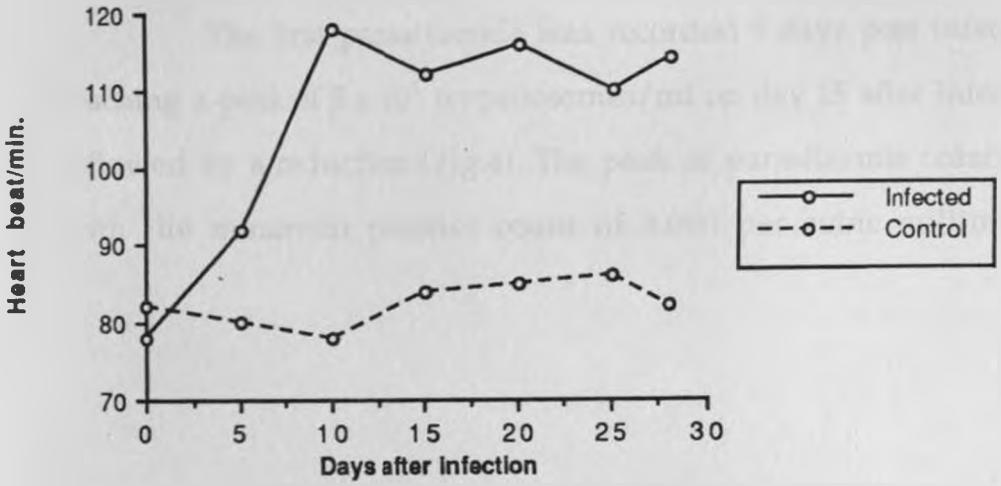
4.1. Clinical Signs

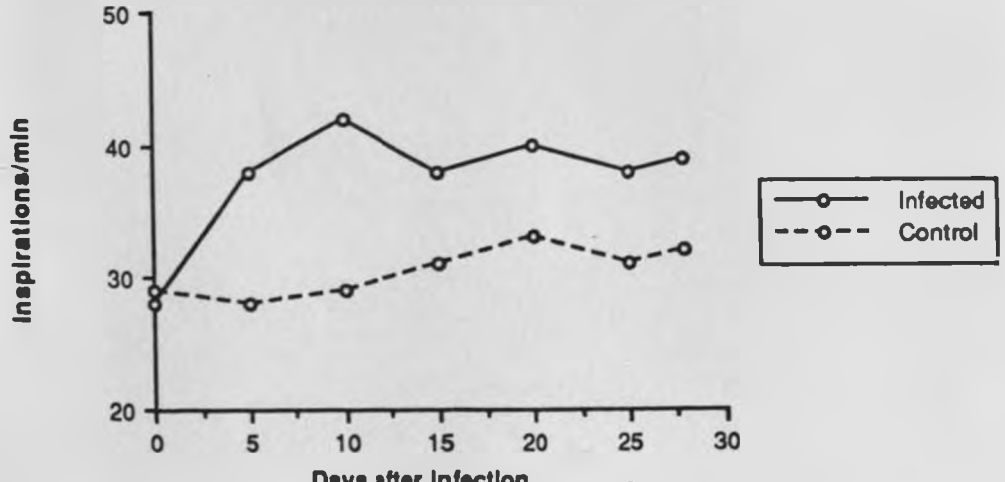
The first rise in body temperature (39.6°C) was recorded on day 5 post infection and the maximum temperature of 40.8°C was recorded on day 10 after infection. The temperature fluctuated throughout the experimental period (Fig.1). The mean range was between 38.5°C and 40.8°C The onset of febrile reaction coincided with the onset of parasitaemia.

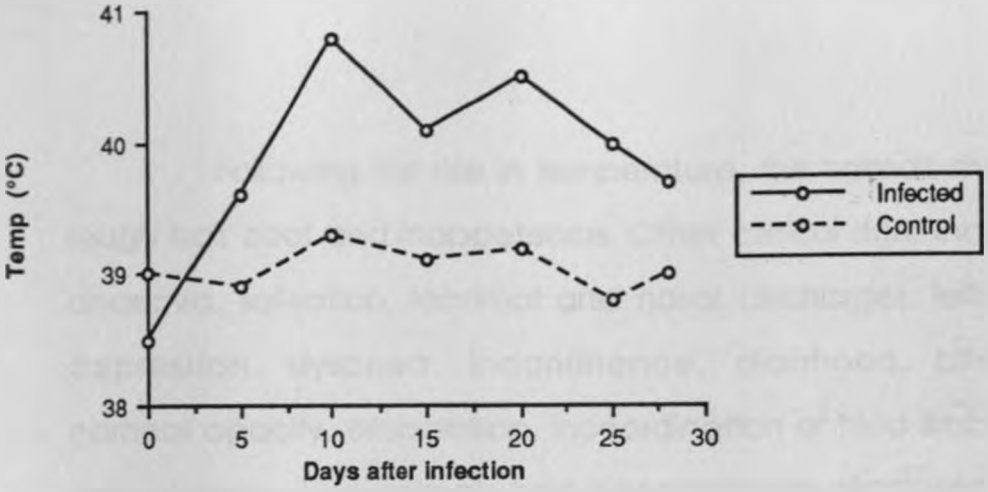
The initial accelerated pulse rate was on day 8 post infection recording 100 beats per minute. As the levels of parasitaemia increased, there was also a corresponding increase in the pulse rate. The highest pulse rate of 118 beats per minute was recorded 10 days after infection (Fig. 2). The mean pulse rate ranged from 78 to 118 beats per minute.

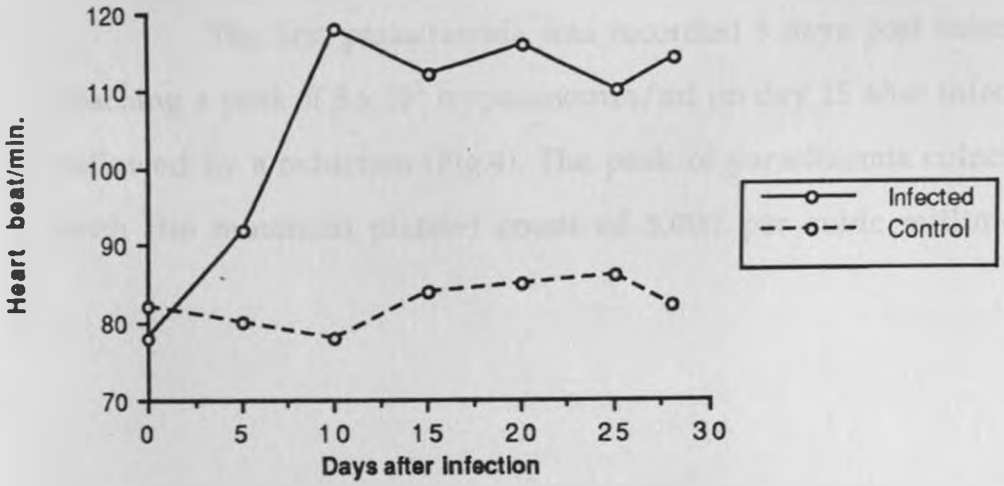
The respiration rate (Fig. 3) began to increase steadily as from day 5 post infection when 38 inspirations per minute were recorded. The mean range was between 28 and 42 inspirations/minute. The highest figure was recorded 10 days post infection. Generally, the increase in respiration rate paralled the increase in temperature, pulse rate and parasitaemia.


Temp (°C)
41
40
39
38
0
5
10
15
20
25
30
Days after infection
Infected
Control


Heart beat/min.
120
110
100
90
80
70
0
5
10
15
20
25
30
Days after infection
Infected
Control


Inspirations/min
50
40
30
20
0
5
10
15
20
25
30
Days after infection.
Infected
Control


Temp (°C)
41
40
39
38
0
5
10
15
20
25
30
Days after infection
Infected
Control


Heart beat/min.
120
110
100
90
80
70
0
5
10
15
20
25
30
Days after infection
Infected
Control

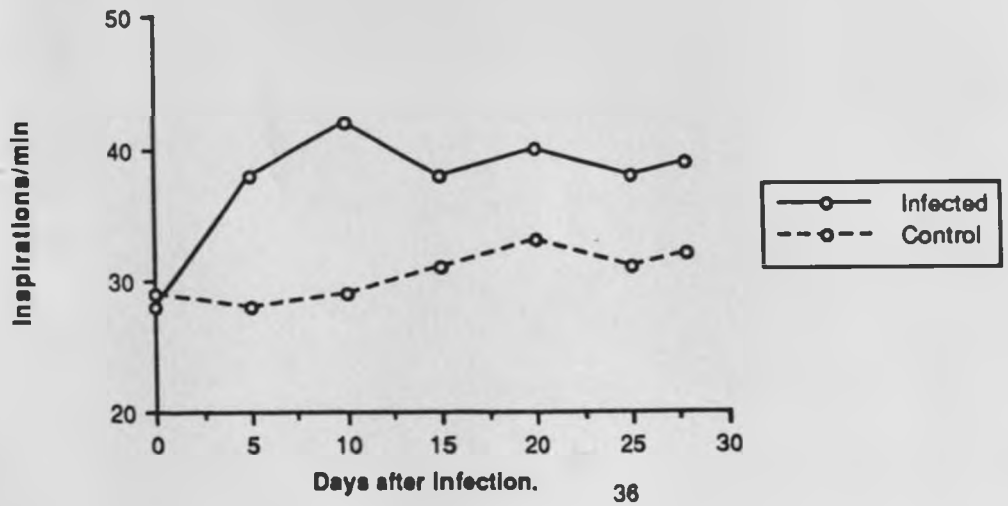
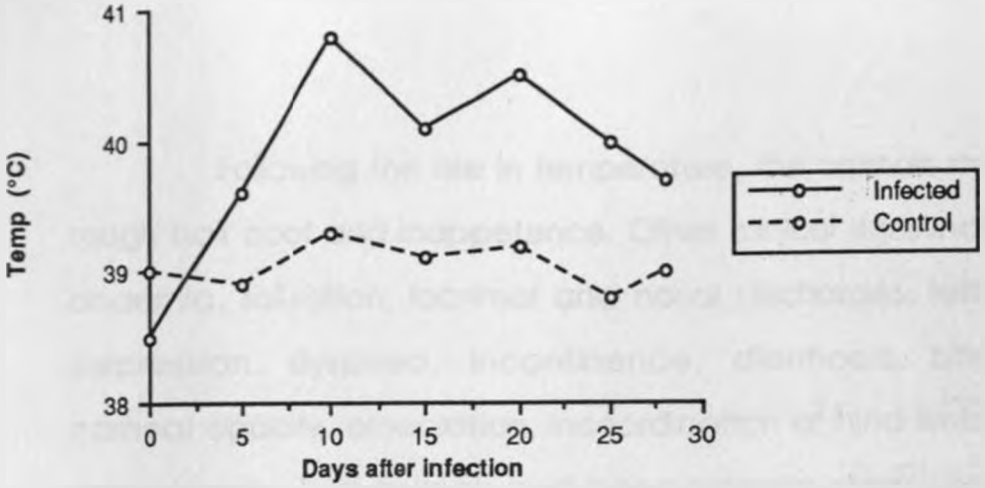
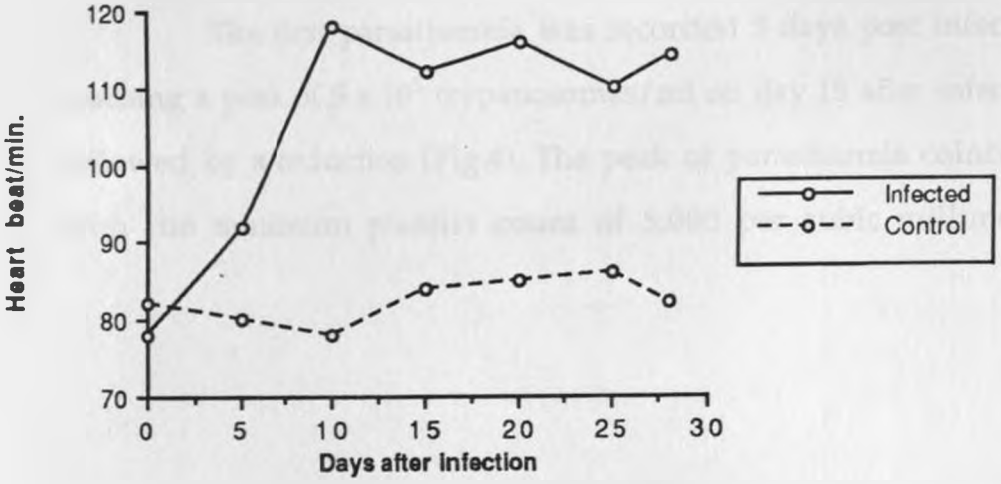

Inspirations/min
50
40
30
20
0
5
10
15
20
25
30
Days after infection.
Infected
Control

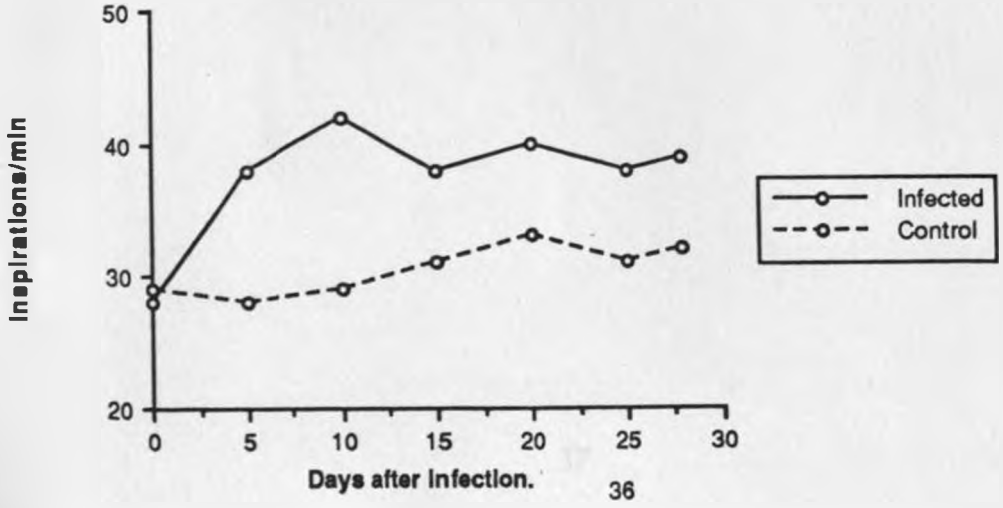
Fig. 1. Temperature (°C) of *T. vivax* infected (o ——— o) and control (o - - - - - o) calves.

Fig. 2. Pulse rate/min. of *T. vivax* infected (o ——— o) and control (o - - - - - - - o) calves.

Fig. 3. Respiration rate/min. of *T. vivax* infected (o ——— o) and control (o - - - - - o) calves.


Temp (°C)
41
40
39
38
0
5
10
15
20
25
30
Days after infection
Infected
Control


Heart beat/min.
120
110
100
90
80
70
0
5
10
15
20
25
30
Days after infection
Infected
Control

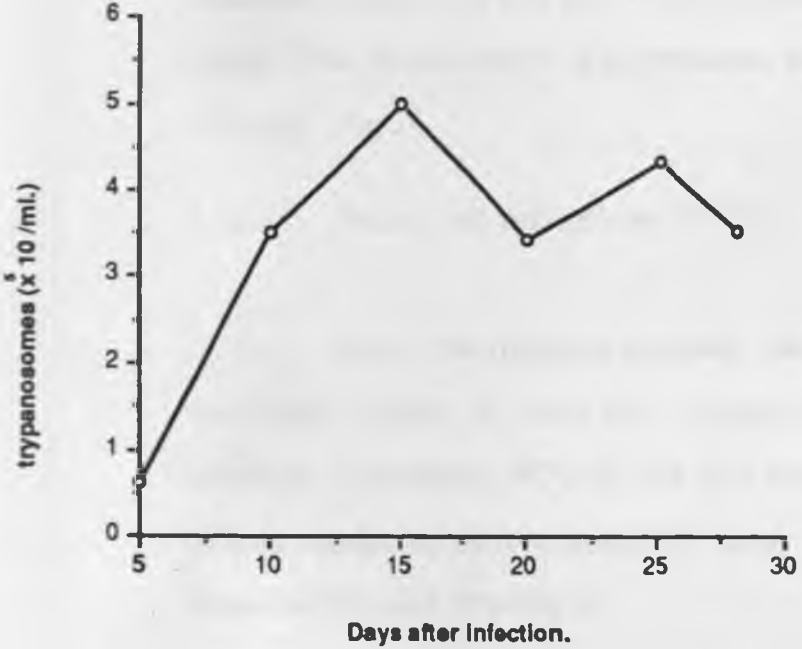

Inspirations/min
50
40
30
20
0
5
10
15
20
25
30
Days after infection.
Infected
Control

Following the rise in temperature, the animals showed rough hair coat and inappetence. Other clinical signs included anaemia, salivation, lacrimal and nasal discharges, lethargy, depression, dyspnea, incontinence, diarrhoea, bilateral corneal opacity, emaciation, incoordination of hind limbs and recumbency. Two animals had haemorrhagic diarrhoea, one died and the other one was sacrificed.

4.2. Parasitological Findings

The first parasitaemia was recorded 5 days post infection reaching a peak of 5×10^5 trypanosomes/ml on day 15 after infection followed by a reduction (Fig.4). The peak of parasitaemia coincided with the minimum platelet count of 5,000 per cubic millimeter.

Fig, 4. Parasitaemia in *T. vivax* infected calves.


trypanosomes ($\times 10^5$ /mL)
0
1
2
3
4
5
6
5
10
15
20
25
30
Days after infection.

4.3. Haematological findings

4.3.1. Red blood cell counts (RBC)

The red blood cell counts began to decrease steadily as from the 10th day after infection up to the end of the experiment, recording the minimum count of 1.9 x $10^6/mm^3$ on day 25 post infection, which is a loss of 71% as compared with the preinfection value. The mean range was between 6.6 x $10^6/mm^3$ and 1.9 x $10^6/mm^3$ (Fig.5).

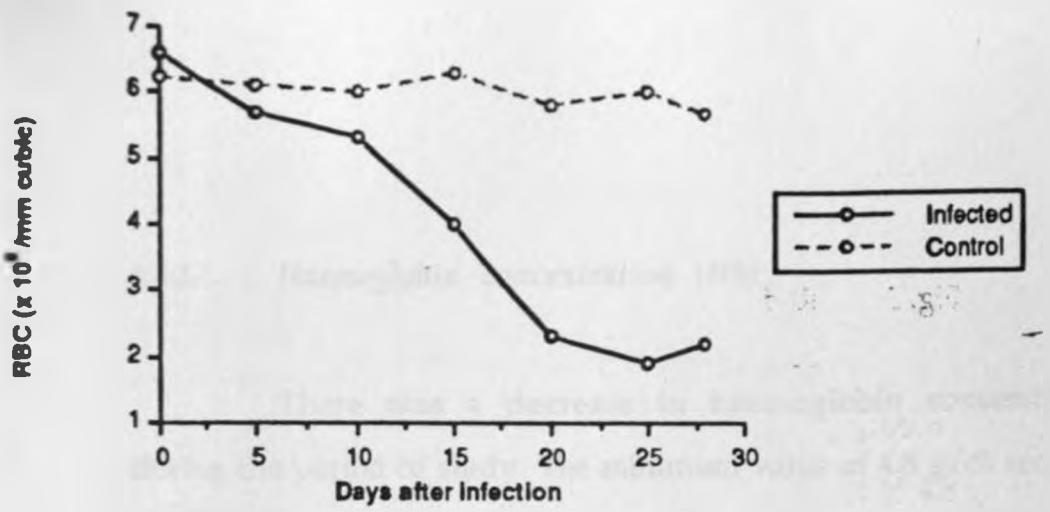
4.3.2. Packed red cell volume (PCV)

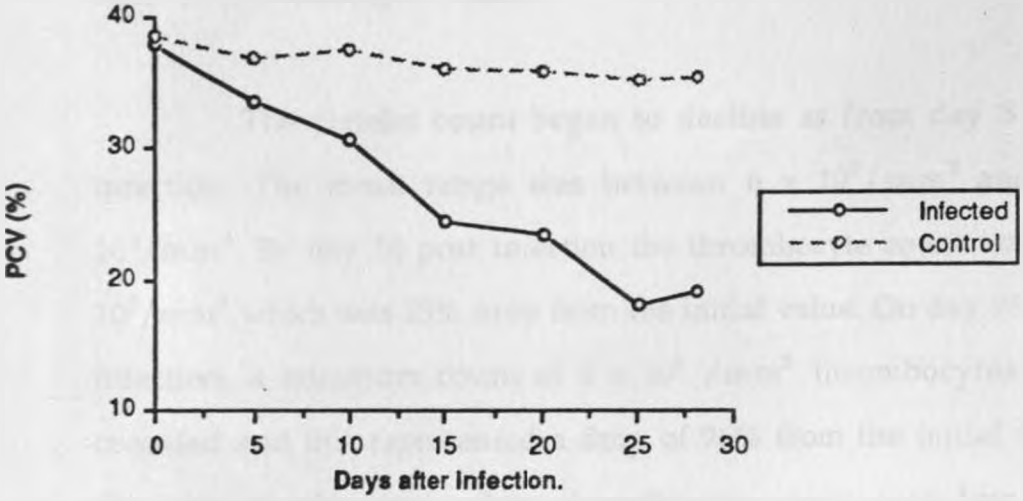
In all the infected animals, the packed red cell volume decreased steadily as from day 10 post infection. On day 25 post infection, a minimum PCV of 18% was recorded which is a drop of 53% as compared with preinfection value. The mean PCV range was between 38% and 18% (Fig.6).

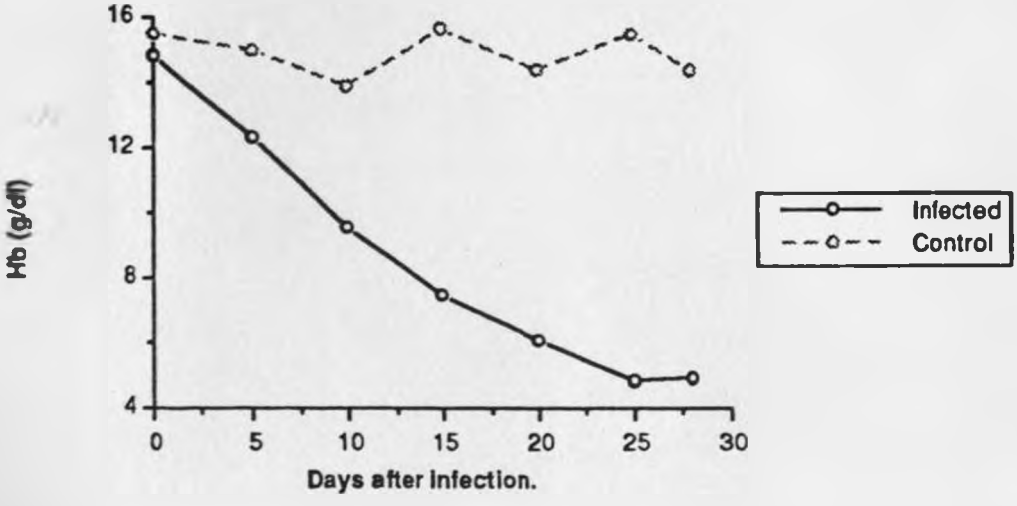
Fig. 5. Red blood cell counts (x 10^6/mm^3 of *T. vivax* infected (o — —— o) and control (o– – – – – – –o) calves.

Fig. 6. Packed red cell volume (PCV%) of *T. vivax* infected (o —— —— o) and control (o– – – – – – –)o calves.

Fig. 7 Haemoglobin concentration (g/dl) of *T. vivax* infected (o - —— o) and control (o – – – – – o) calves.


RBC (x 10⁶ /mm cubic)
7
6
5
4
3
2
1
0
5
10
15
20
25
30
Days after infection
Infected
Control


PCV (%)
40
30
20
10
0
5
10
15
20
25
30
Days after infection.
Infected
Control


Hb (g/dl)
16
12
8
4
0
5
10
15
20
25
30
Days after infection.
Infected
Control

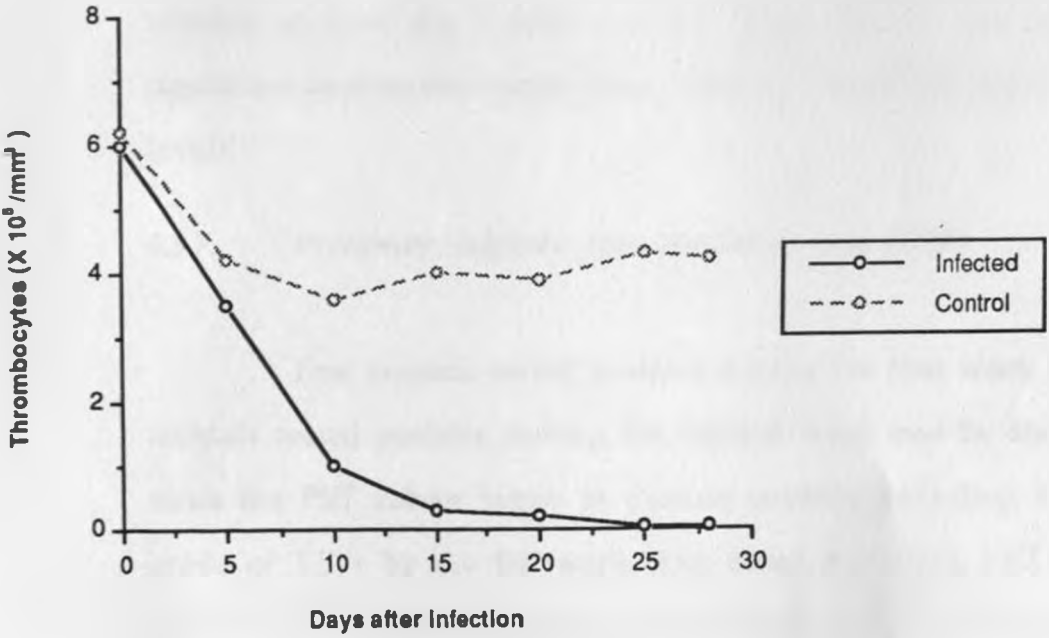
4.3.3. *Haemoglobin concentration (Hb)*

There was a decrease in haemoglobin concentration during the period of study. The minimum value of 4.8 g/dl recorded on day 25 after infection was a drop of 66% as compared with preinfection value and that of the controls (Fig. 7).

4.3.4 *Thrombocyte counts.*

The platelet count began to decline as from day 5 post infection. The mean range was between 6 x $10^5/mm^3$ and 5 x $10^3/mm^3$. By day 10 post infection the thrombocyte count was 1 x $10^5/mm^3$ which was 25% drop from the initial value. On day 25 post infection, a minimum count of 5 x 10^3 $/mm^3$ thrombocytes was recorded and this represented a drop of 96% from the initial value (Fig. 8). At the time when thrombocyte count was low, the parasitaemia levels were high.

Fig. 8. Thrombocyte counts (x $10^5/mm^3$ of *T. vivax* infected (o — —— o) and control (o – – – – – o) calves.


Thrombocytes (X 10⁵ /mm³)
8
6
4
2
0
0
5
10
15
20
25
30
Days after infection
Infected
Control

4.3.5. *Plasma protein concentration (PPC)*

There was slight but not significant decrease ("t" test at 5% significance level) in the total plasma protein concentration as the values were still within the normal range (Fig. 9).

4.3.6. *Plasma fibrinogen concentration (PFC)*

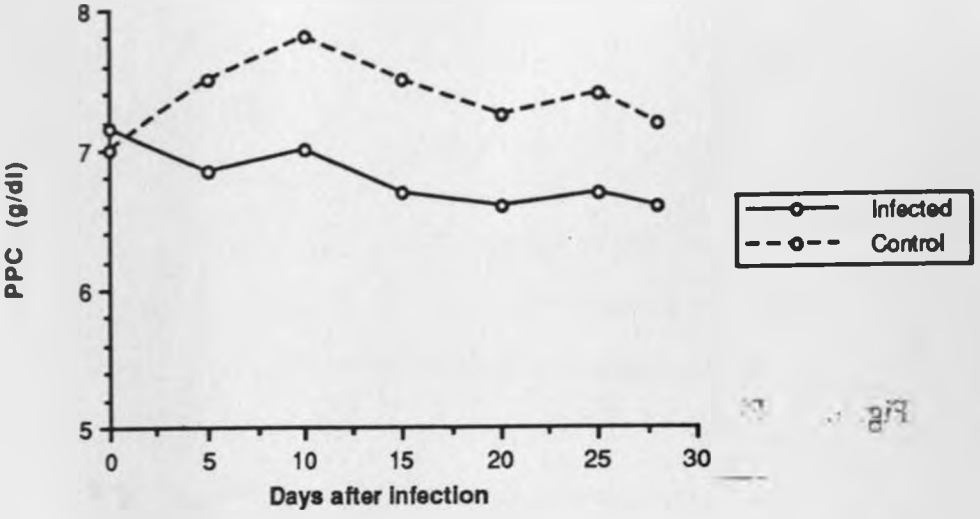
The plasma fibrinogen concentration showed a slight increase as from day 5 post infection (Fig. 10), but this was not significant as compared with the controls ("t" test at 5% significance level).

4.3.7. *Protamine sulphate paracoagulation test (PST)*

Few animals tested positive during the first week but all animals tested positive during the second week and by the third week the PST values began to decline steadily recording a mean grade of 1.5 + by the 4th week. The mean maximum PST (4.5 + grade) coincided with the high parasitaemia, pyrexia and low platelet count (Fig. 11).

Fig. 9. Plasma protein concentration (g/dl) in *T. vivax* infected (o —— o) and control (o - - - - - o) calves.

Fig. 10. Plasma fibrinogen concentration (g/dl) in *T. vivax* infected (o —— o) and control (o - - - - - o) calves.


PPC (g/dl)
8
7
6
5
0
5
10
15
20
25
30
Days after infection
Infected
Control

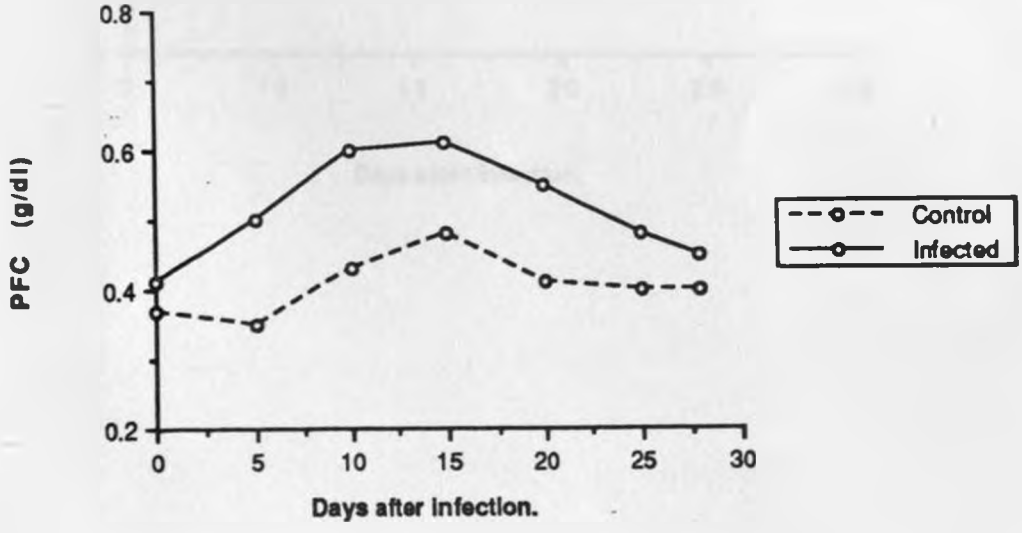
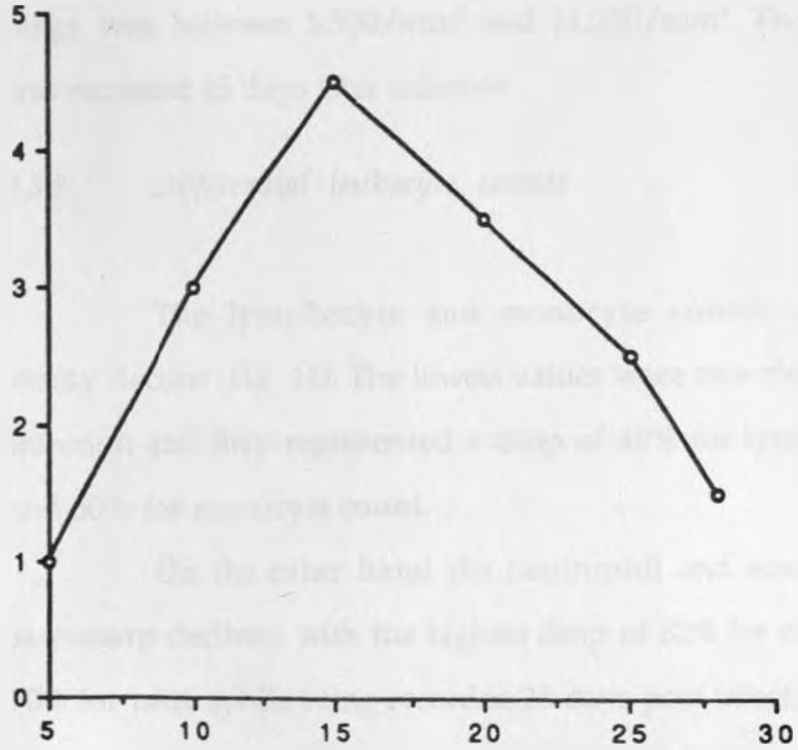

PFC (g/dl)
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
Days after infection.
Control
Infected

Fig. 11. P S T in *T. vivax* infected calves.


PST (GRADE +)
5
4
3
2
1
0
5
10
15
20
25
30
Days after infection.

4.3.8. *White blood cell counts (WBC)*

The total white blood cell counts showed a steady decline during the first two weeks of infection followed by a steady rise which surpassed the initial preinfection value and that of the controls, towards the end of the experiment (Fig. 12). The mean range was between 5,500/mm^3 and 14,000/mm^3. The lowest count was recorded 15 days after infection.

4.3.9. *Differential leukocyte counts*

The lymphocyte and monocyte counts experienced a steady decline (Fig. 13). The lowest values were recorded 28 days post infection and they represented a drop of 40% for lymphocyte count and 50% for monocyte count.

On the other hand the neutrophil and eosinophil counts saw sharp declines, with the highest drop of 82% for eosinophils and 70% for neutrophils being recorded 28 days post infection.

Fig. 12. White blood cells counts (x 10^3/mm^3 of *T. vivax* infected (o ——— o) and control (o – – – – – o) calves.

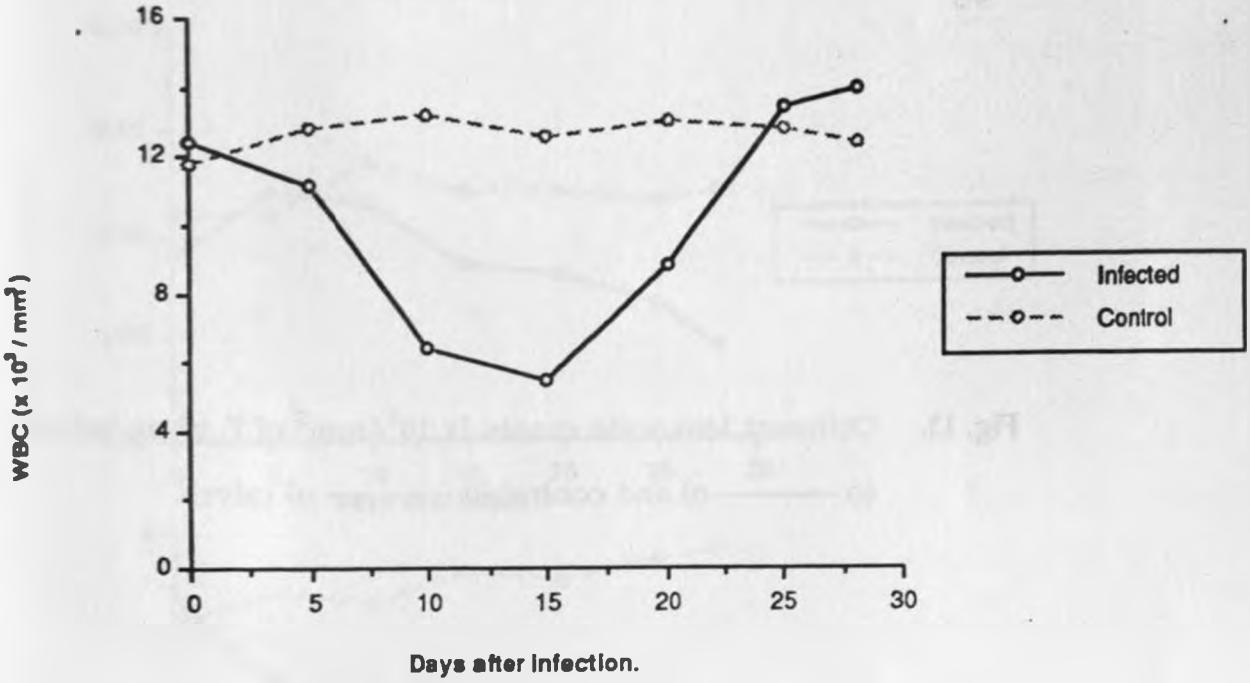
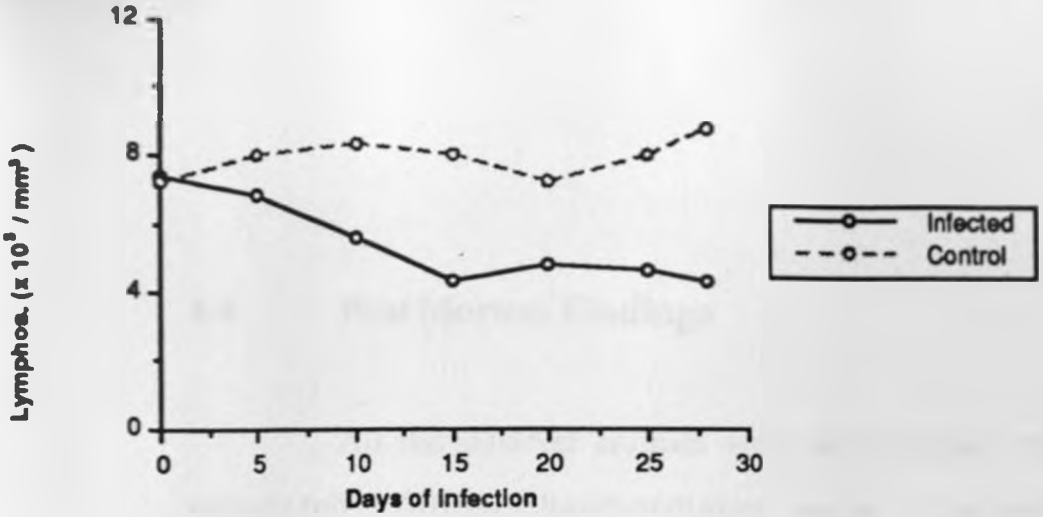
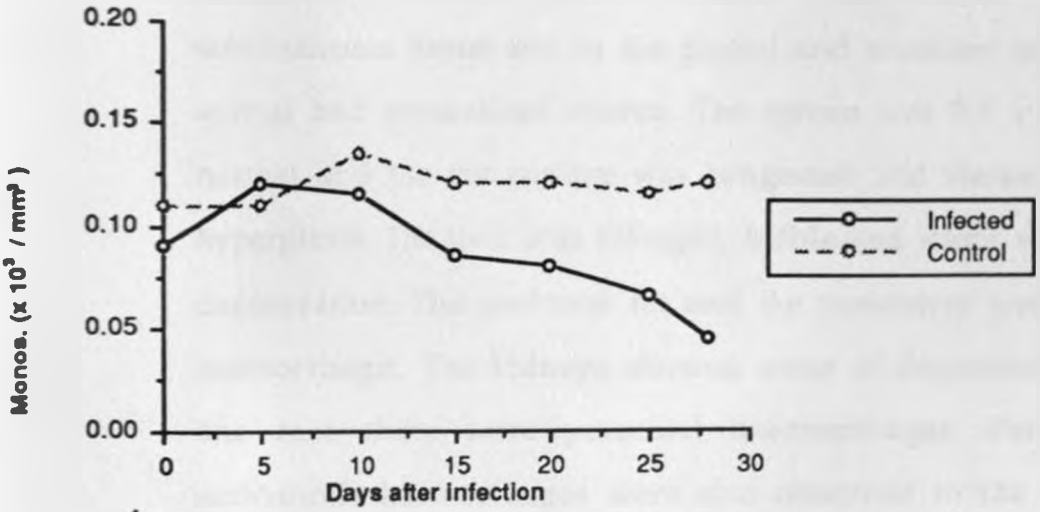
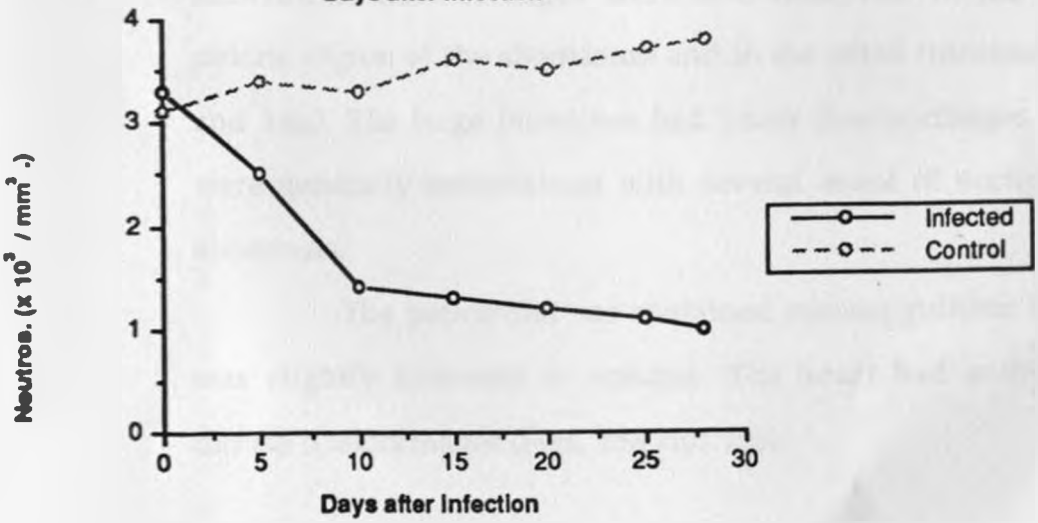
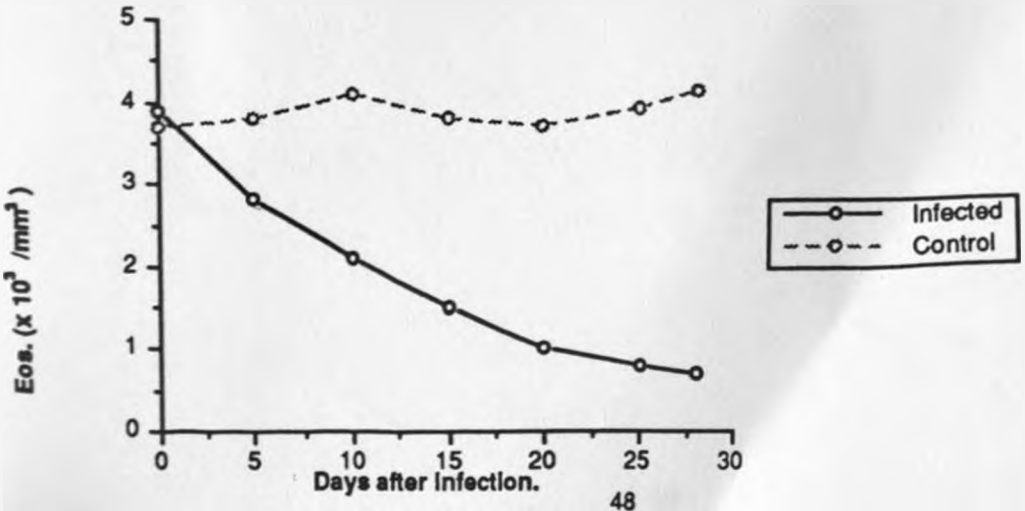

16
12
8
4
0
0
5
10
15
20
25
30
WBC (x 10³ / mm³)
Days after infection.
Infected
Control

Fig. 13. Different leukocyte counts (x $10^3/mm^3$ of *T. vivax* infected (o ——— o) and control (o - - - - - o) calves.


Lymphoc. (x 10³ / mm³)
12
8
4
0
0
5
10
15
20
25
30
Days of infection
Infected
Control


Monos. (x 10³ / mm³)
0.20
0.15
0.10
0.05
0.00
0
5
10
15
20
25
30
Days after infection
Infected
Control


Neutros. (x 10³ / mm³.)
4
3
2
1
0
0
5
10
15
20
25
30
Days after infection
Infected
Control


Eos. (x 10³ /mm³)
5
4
3
2
1
0
0
5
10
15
20
25
30
Days after infection.
Infected
Control

4.4 Post Mortem Findings

All the infected animals were dehydrated, anaemic and emaciated. Diffuse haemorrhages were observed in the subcutaneous tissue and in the gluteal and shoulder muscles. One animal had generalized icterus. The spleen was 2-3 x as large as normal and the cut surface was congested and showed follicular hyperplasia. The liver was enlarged, friable and icteric with areas of degeneration. The perirenal fat and the mesentery were diffusely haemorrhagic. The kidneys showed areas of degeneration and in one case there were petechial haemorrhages. Petechial and ecchymotic haemorrhages were also observed in the edematous pyloric region of the abomasum and in the small intestines (Figs. 14a and 14b). The large intestines had linear haemorrhages. The lungs were generally oedematous with several areas of ecchymoses and atelectasis.

The pericardial sac contained serosanguinous fluid which was slightly increased in volume. The heart had ecchymotic and diffuse haemorrhages (Figs. 15a and 15b).

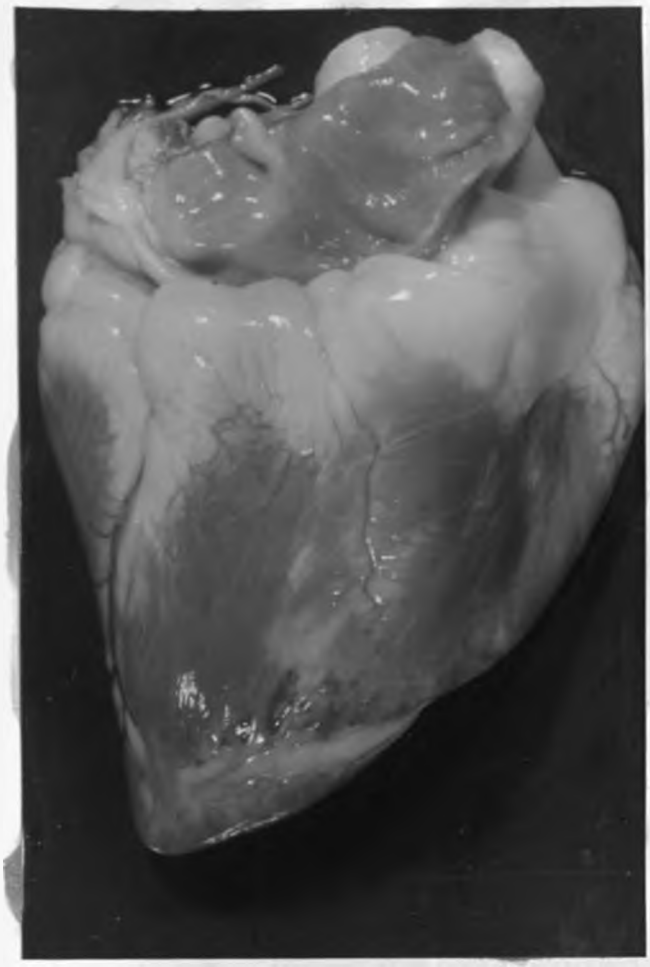
Fig. 14 a). Abomasum of *T. vivax* infected steer K 124 showing petechial haemorrhage and oedema.

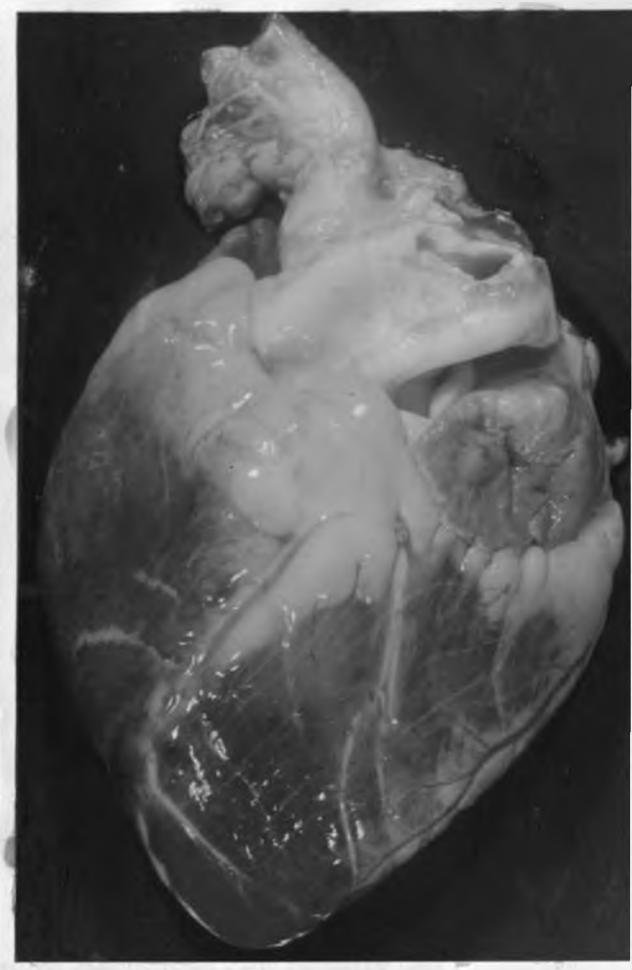
Fig 14 b). Large and small intestines of *T. vivax* infected steer K 124 showing linear and petechial haemorrhages.

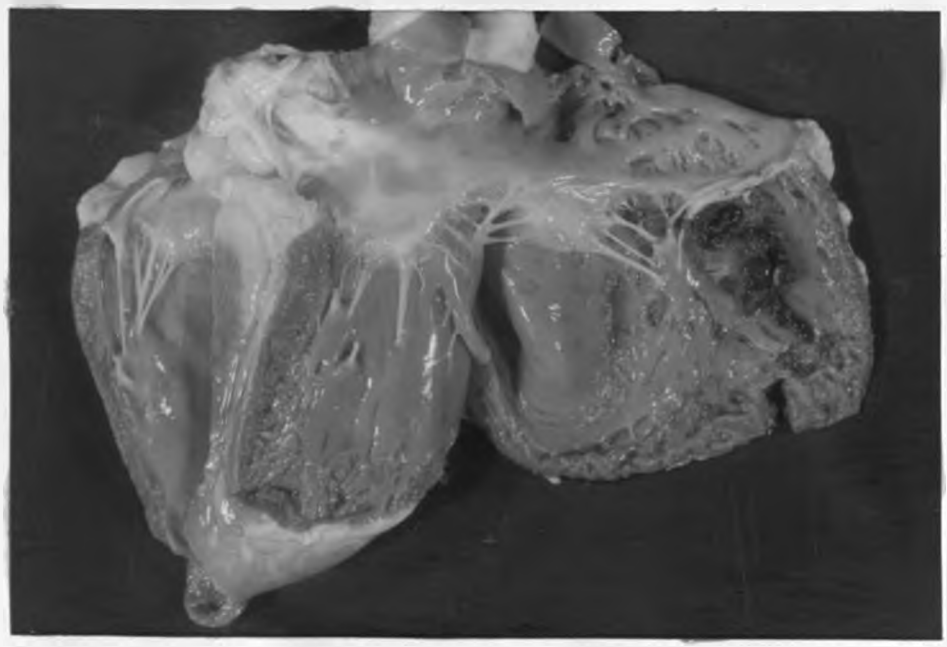
Fig. 15 a). Heart of *T. vivax* infected bull calf K 128 showing petechial haemorrhage.

Fig. 15 b). Heart of *T. vivax* infected bull calf K 130 showing diffuse haemorrhage.

Fig. 15 c). Heart of *T. vivax* infected bull calf K 128 showing a thrombus in the right ventricle.







The diffuse haemorrhages were more marked in the right ventricle and atria than in the left side of the heart. In two cases, thrombi were observed in the right ventricle (Fig. 15c). The lymph nodes were enlarged and oedematous. The bone marrow of the long bones was activated except for three animals. The brain and the spinal cord did not show any significant gross lesions.

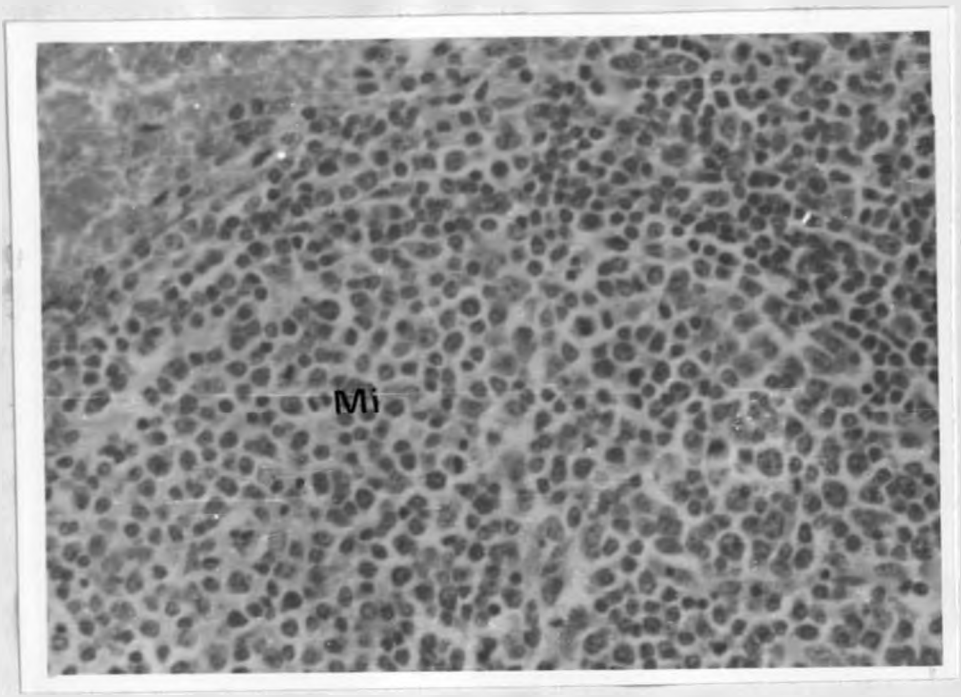
4.5. Histopathology

4.5.1. Spleen

The spleen had an increase in the number and size of the follicles with marked activity of the germinal centres. In one case, the germinal centres were widened with decreased cell density, an indication of partial depletion of the lymphoid cells. Within the follicles were lymhocytes, lymphoblasts and macrophages, and many of these cells had mitotic figures (Fig. 16). Plasma cells had infiltrated the periarteriolar lymphoid sheath and the red pulp. The red pulp was also hyperemic and haemorrhagic, with hemosiderosis, erythrophagocytosis, and presence of megakaryocytes (Fig. 17). Macrophages, lymphoytes and precursor cells of erythropoiesis were also observed in the red pulp. The capillaries and the sinusoides contained lymphoid cells.

Fig. 16. Histological section of spleen of *T. vivax* infected steer K 121. The follicle shows proliferation of lymphoid cells with mitotic figures (Mi). H.E. x 400.

Fig. 17. Histological section of spleen of *T. vivax* infected steer K 121 showing atypical megakaryocyte (Me) in the medullary region. Lymphoid cells have also infiltrated the area. T = Trabeculum. H.E. x 400.


Mi

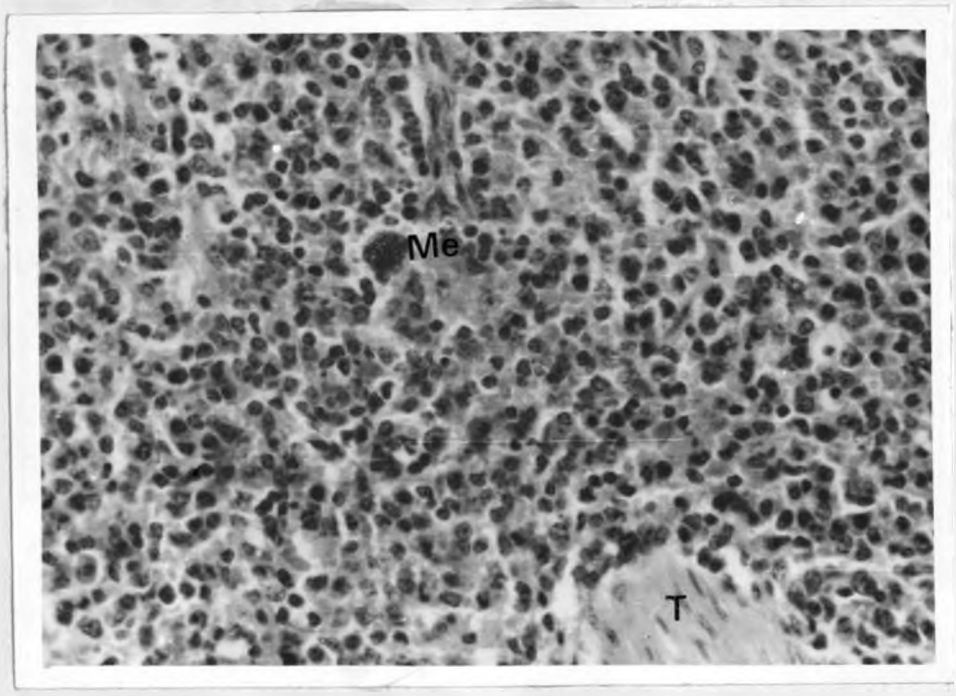
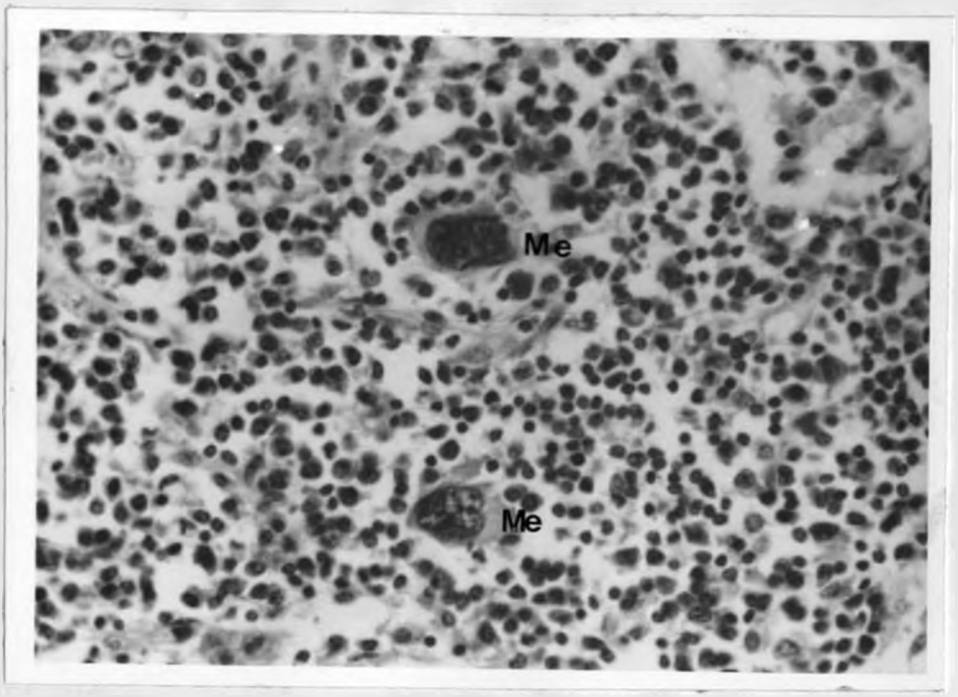
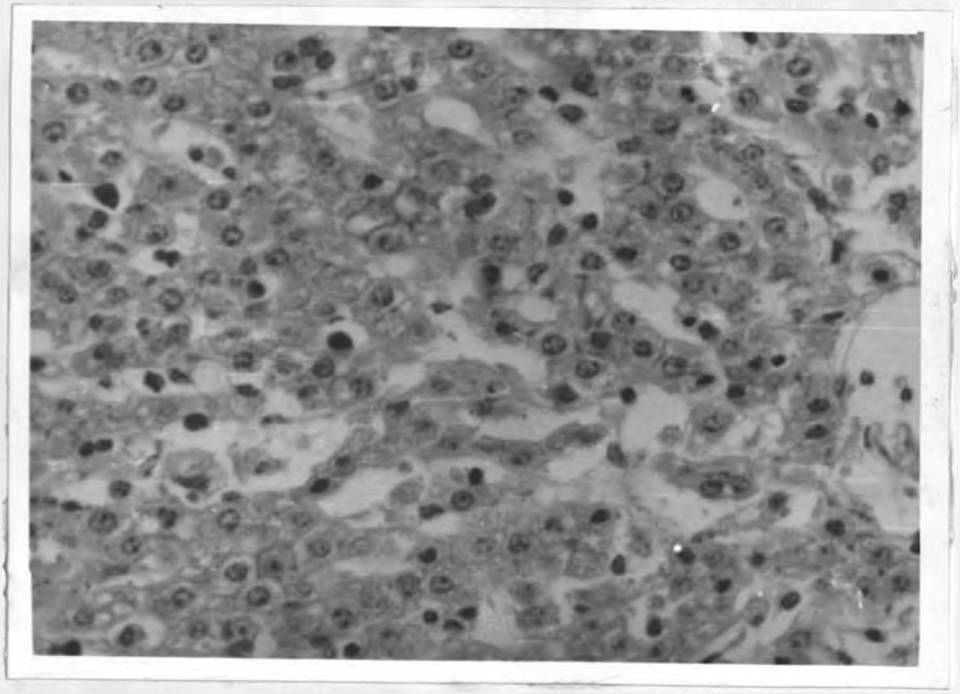

Me
T

Fig. 18. Histological section of lymph node of *T. vivax* infected steer K 123 showing two megakaryocytes (Me) and lymphoid cells in the medullary region. H.E. x 400.

Fig. 19. Histological section of liver of *T. vivax* infected steer K 123 showing vacuolar degeneration and prominence of Kupffer cells. H.E. x 400.


Me
Me



4.5.4. *Heart*

In all the infected animals, there were subepicardial, myocardial and subendocardial haemorrhages (Fig. 20). Lymphocytes, plasma cells and macrophages had infiltrated the epicardium, endocardium and myocardium (Fig. 21). The myofibres were fragmented, atrophic and widely separated and some were myolytic. Thrombi were observed in some blood vessels (Fig. 22). Purkinje cells were also fragmented.

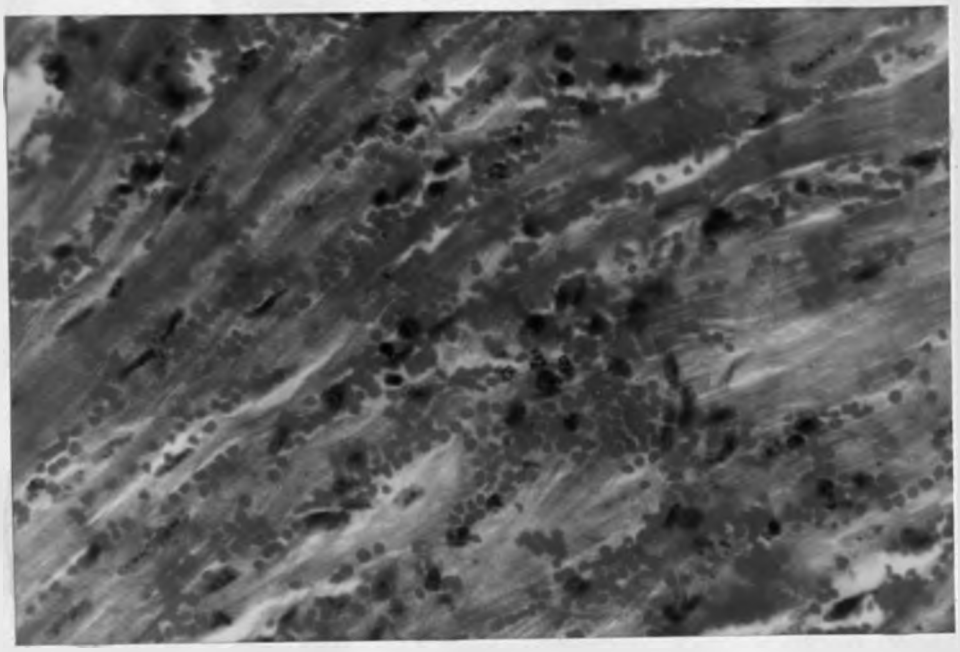
4.5.5. *Kidney*

Renal lesions included multifocal perivascular, periglomerular and interstitital lymphoid cell infiltrations (Fig. 23).

Tubular nephrosis and presence of homogenous proteinaceous material were also observed (Fig. 24). Focal haemorrhages were observed in few cases.

Fig. 20 Histological section of myocardium of *T. vivax* infected bull calf K 129 showing haemorrhage and lymphoid cell infiltration. Masson's trichrome x 400.

Fig. 21. Histological section of myocardium of *T. vivax* infected steer K 121 showing fragmentation and lysis of myofibres (Mf), haemorrhage and lymphoid cell infiltration (L). E = Erythrocyte. H.E. x 400.



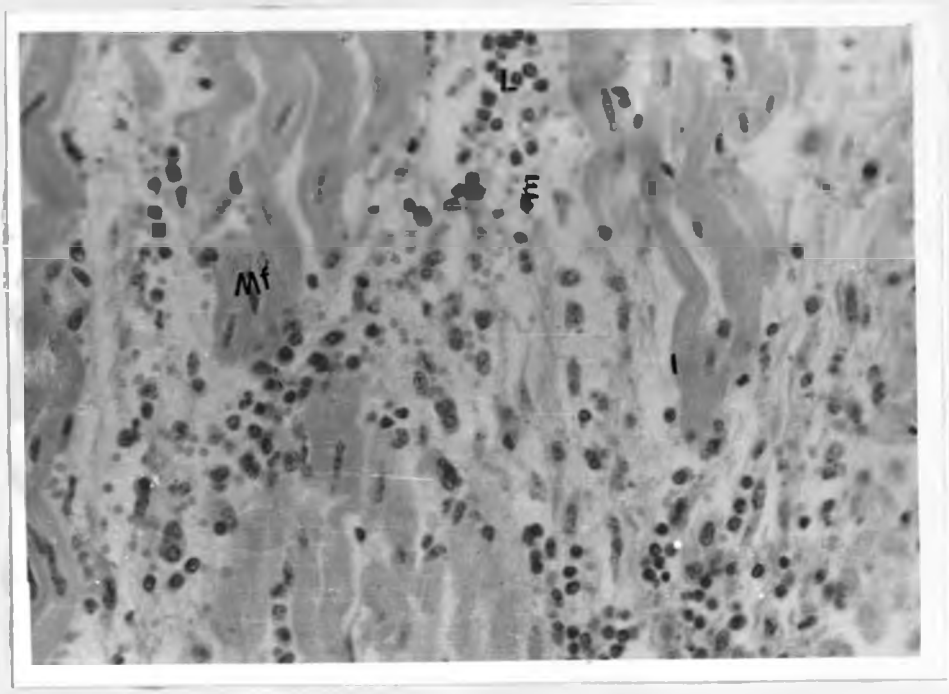
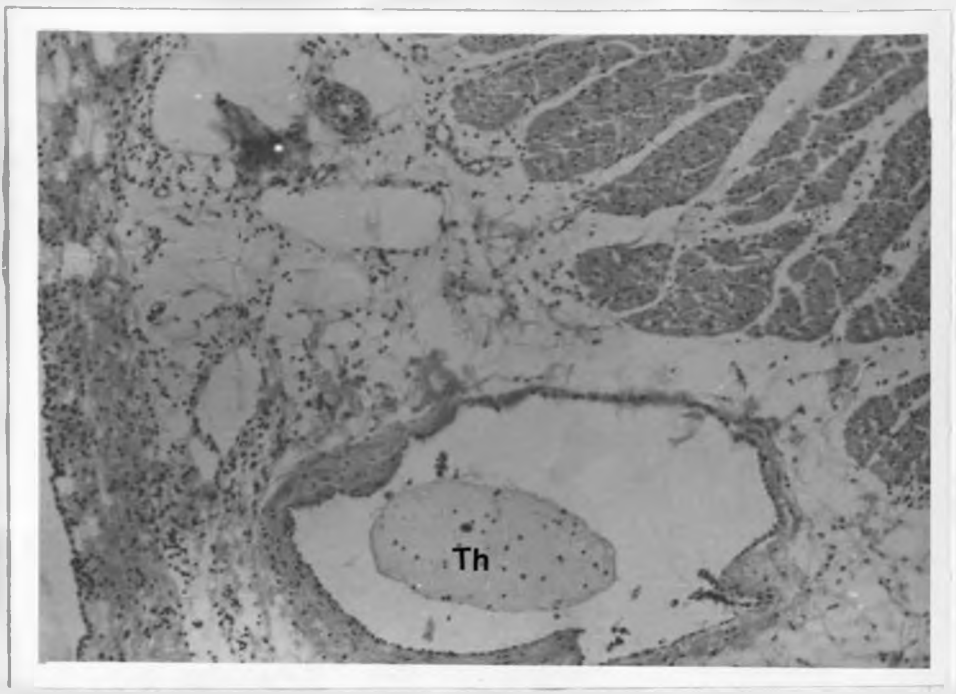
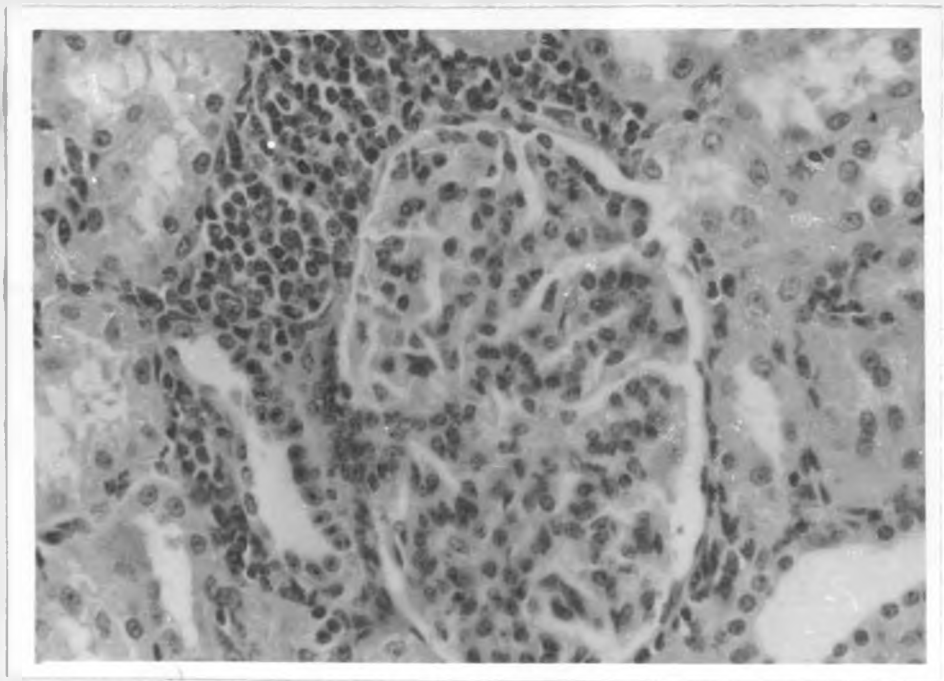

Mf
Mf

Fig. 22. Histological section of myocardium of *T. vivax* infected steer K 123 showing lymphoid cell infiltration and hyaline thrombus (Th). H.E. x 100.

Fig 23. Histological section of kidney of *T. vivax* infected steer K 121 showing interstitial and glomerular lymphoid cell infiltration H.E. x 400.


Th



4.5.6. *Lungs*

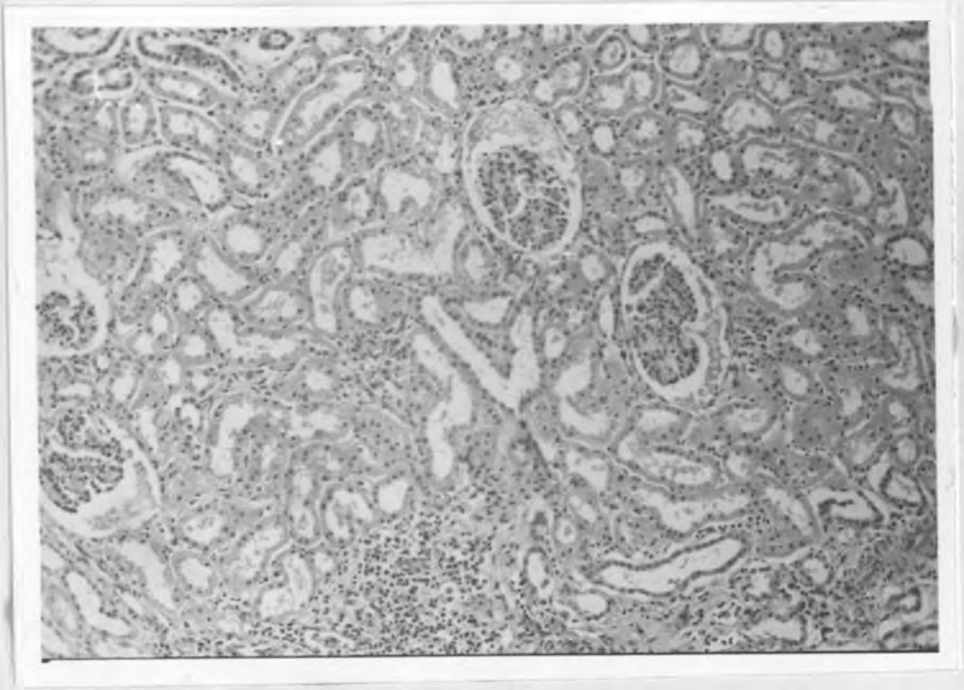
The lungs were congested and some showed interstitial and alveolar oedema (Fig. 25). Lymphocytes had infiltrated the perivascular, peribronchial and interstitial tissue. Atypical megakaryocytes were seen in the alveolar septa (Fig. 26).

4.5.7. *Abomasum*

Submucosal oedema was a common lesion in all the infected animals. The blood and lymph vessels were dilated (Fig. 27) and there was disruption of the collagen and reticular fibres. Lymphoid and plasma cells had infiltrated the submucosal and subglandular areas.

Fig 24. Histological section of kidney of *T. vivax* infected steer K 123 showing proteinaceous material in the glomerular space, and interstitial lymphoid cell infiltration H.E. x 100

Fig. 25. Histological section of lung of *T. vivax* infected steer K 122 showing alveolar and interstitial oedema. H.E. x 400



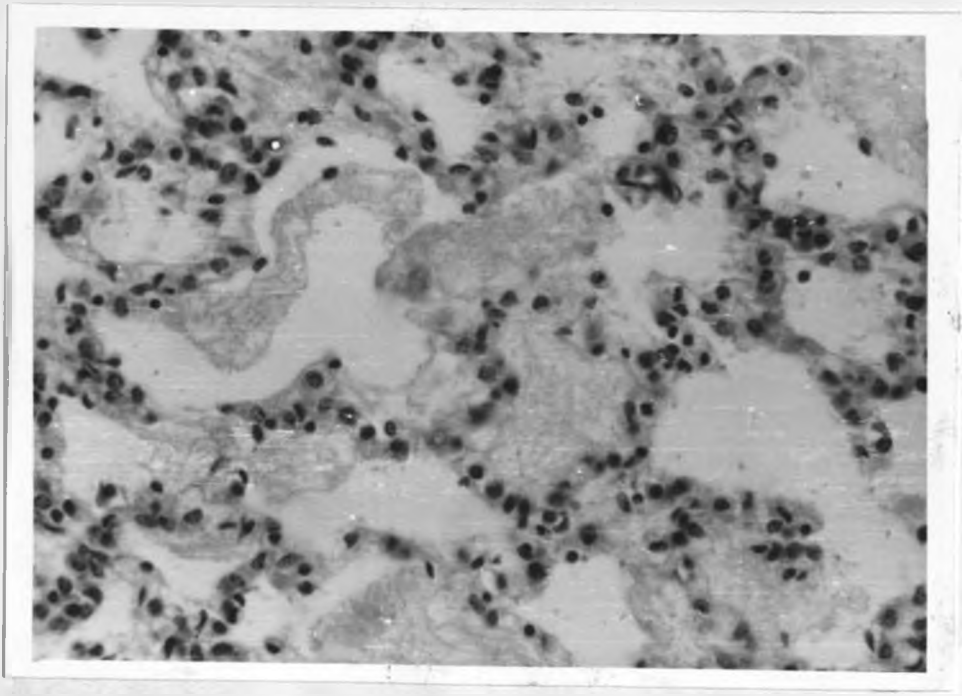
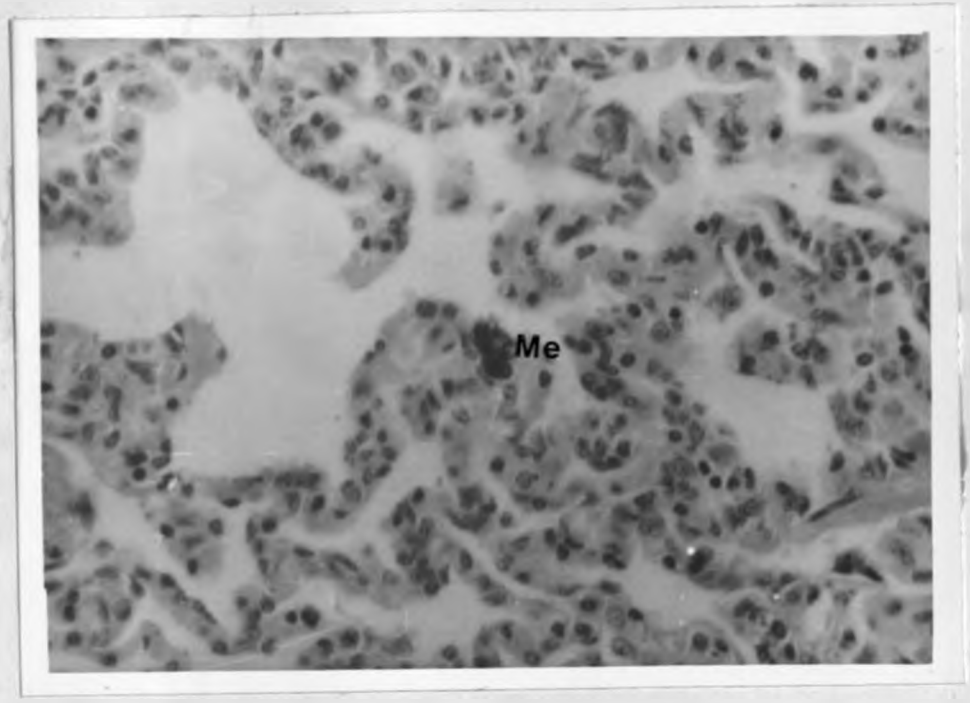
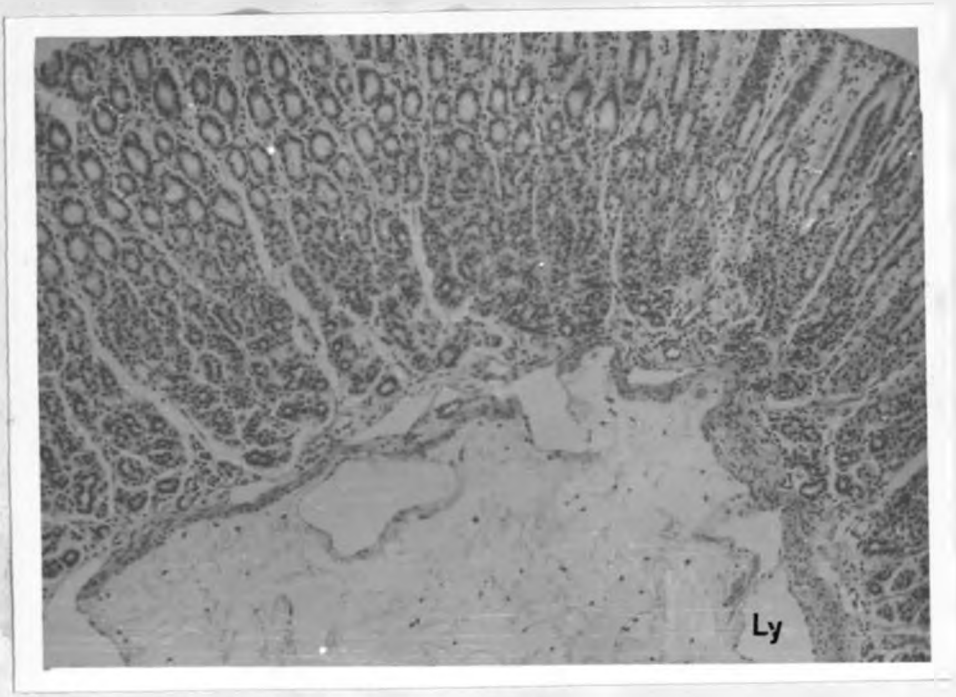


Fig. 26. Histological section of lung of *T. vivax* infected steer K 121 showing atypical megakaryocyte (Me) in the alveolar septum. H.E. x 400.

Fig. 27. Histological section of abomasum of *T. vivax* infected steer K 121 showing submucosal oedema. The blood vessels and lymphatics (Ly) are dilated. There is also subglandular lymphoid cell infiltration H.E. x 100.


Me


Ly

4.5.8. Brain

The brain showed perivascular lymphoid cell infiltration (Fig. 28) in animals that were killed 28 days post infection. Those that were killed 13 and 22 days post infection did not show the lesion.

4.5.9. Blood vessels

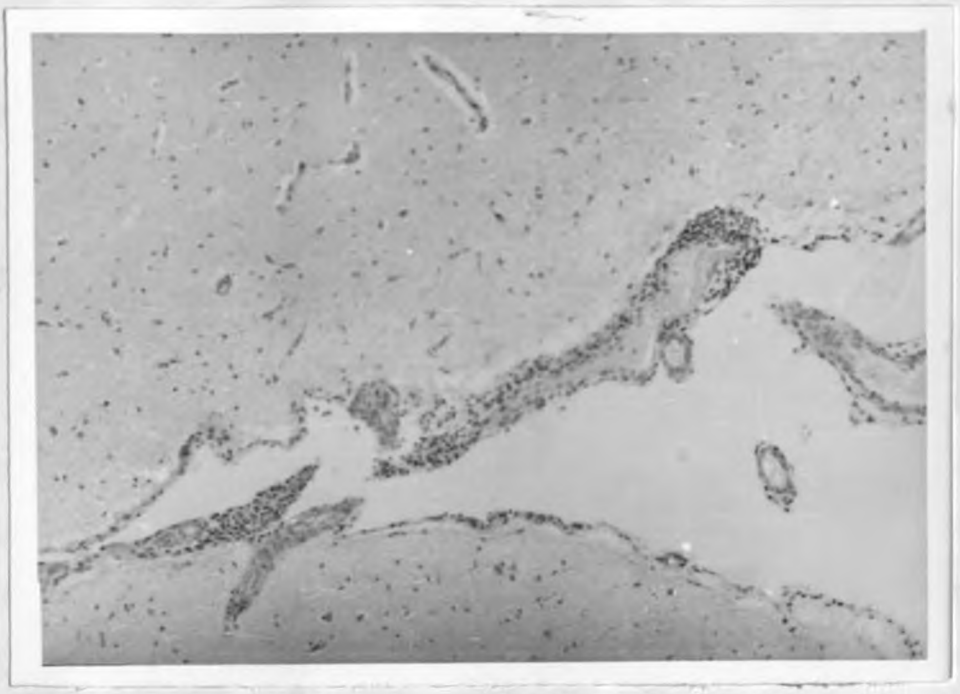
The capillaries were ruptured leading to haemorrhages (Fig. 29) and the ruptured blood vessels contained no blood cells. Some blood vessels were congested and some contained fibrin and hyaline thrombi (Fig. 22).

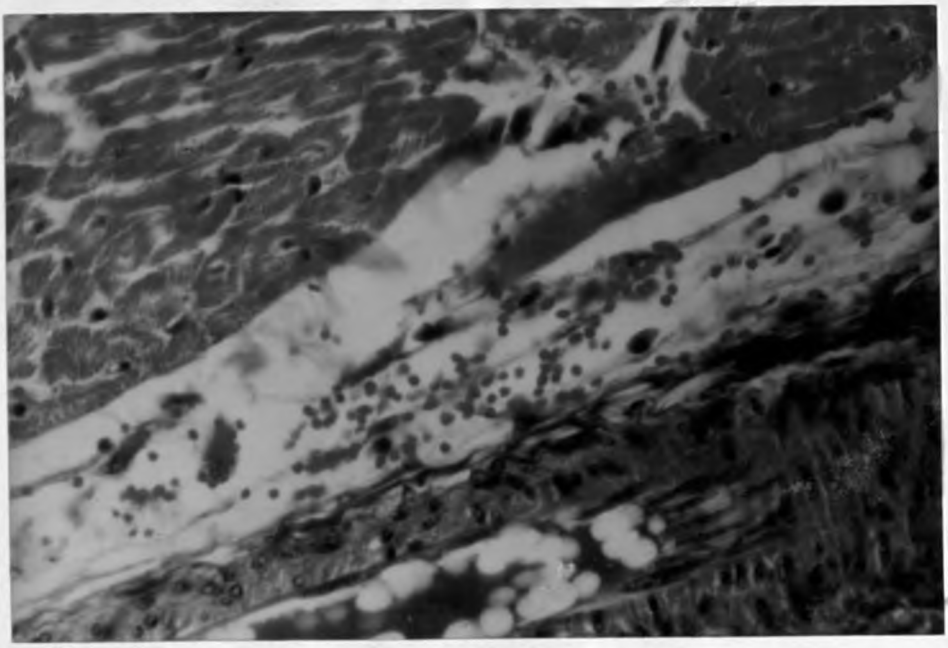
4.5.10. Bone marrow

The sternal marrow biopsy taken 9 days after infection had a myeloid: erythroid (M:E) ratio of 0.75. The second one taken 16 days after infection had a M.E. ratio of 0.83 and the one taken 22 days post infection had a M:E ratio of 0.92. The megakaryocytes on day 9 post infection accounted for 0.42% of the marrow cells and 0.5% on day 22 post infection. 54% of the megakaryocytes had low nucleus: cytoplasma ratio, 4% had hyperchromatic nuclei. Most of the nuclei were pyknotic and hyperchromatic but some showed polyploidy with little cytoplasm. Some megakaryocytes and macrophages showed intracytoplasmic vacuolation and cellular phagocytosis. There was generally increased cellularity in the marrow (Fig. 30).

Fig. 28. Histological section of brain of *T. vivax* infected steer K 121 showing meningeal and perivascular cell infiltration. H.E. x 100.

Fig. 29. Histological section of myocardium of *T. vivax* infected bull calf K 128 showing ruptured blood vessel and haemorrhage. Masson's trichrome. x 400.





4.6 Electron Microscopy

4.6.1. Platelets

The platelets examined up to 7 days post infection had no change. All the organelles were present and alpha, beta, delta and gamma granules were quite visible (Figs. 31 and 32). On day 13 post infection, some platelets had vacuoles and slight degranulation (Fig. 33) which continued in intensity until 22 days after infection when K 123, 124 were killed (Figs. 34, 35, 36). The platelets of steer K 121, however, showed no degranulation on day 28 post infection (Fig. 37) when the animal was sacrificed.

Fig. 30. Histological section of bone marrow of *T. vivax* infected steer K 121 showing increased cellularity. Megakaryocytes (Me) are at different stages of maturation. H.E. x 100.

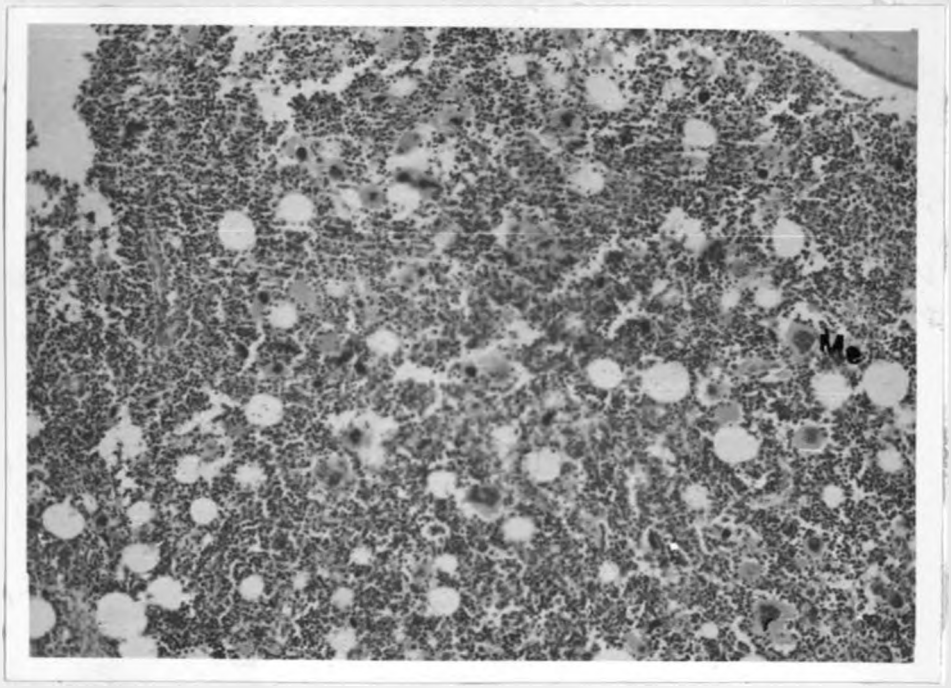
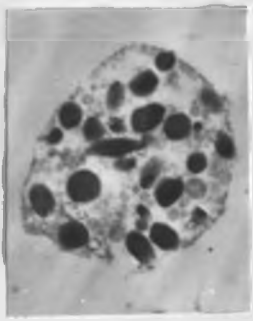
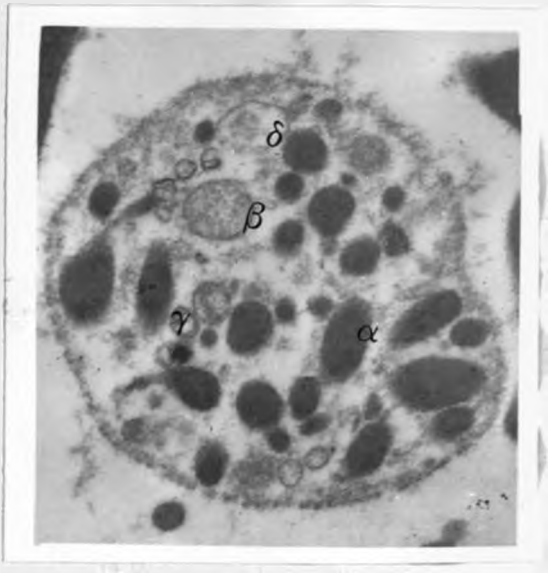

Me

Fig. 31. Electron micrograph of a platelet of *T. vivax* infected steer K 121, 6 days after infection showing intact granules. x 13,800.

Fig. 32. Electron micrograph of a platelet of *T. vivax* infected steer K 123, 11 days post infection showing alpha, beta, delta and gamma granules x 27,000.

Fig. 33. Electron mycrograph of a platelet of *T. vivax* infected steer K 123, 13 days post infection showing degranulation and vacuolation (v). x 13,800.




δ
β
γ
α

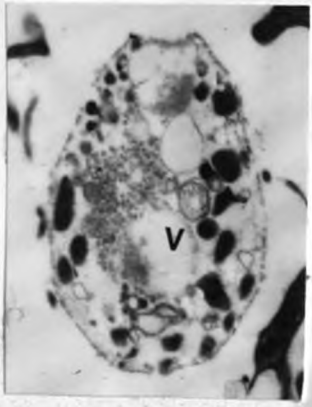
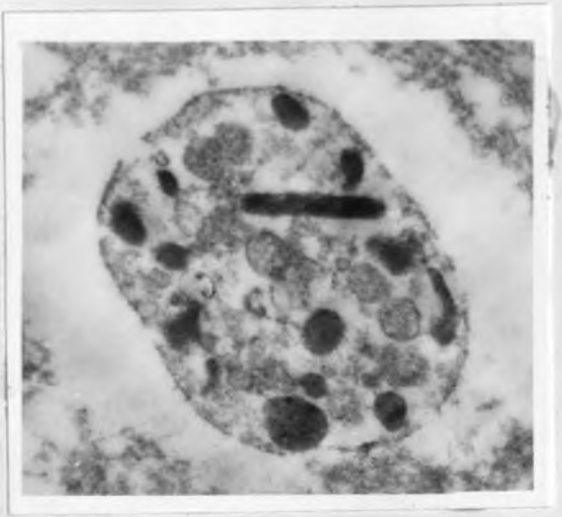
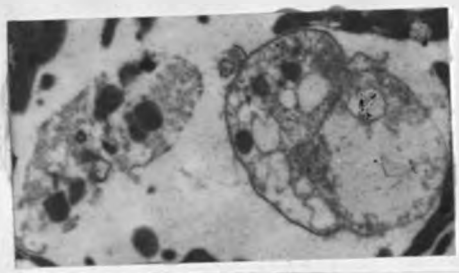

V

Fig. 34 Electron micrograph of a platelet of *T. vivax* infected steer K 123, 15 days post infection showing degranulation. x 27,000.

Fig. 35. Electron micrograph of platelets of *T. vivax* infected steer K 123, 18 days post infection showing degranulation and vacuolation x 5,700.

Fig. 36. Electron micrograph of platelets of *T. vivax* infected steer K 124, 24 days post infection showing degranulation of some platelets. P S = pseudopodium x 13,800.





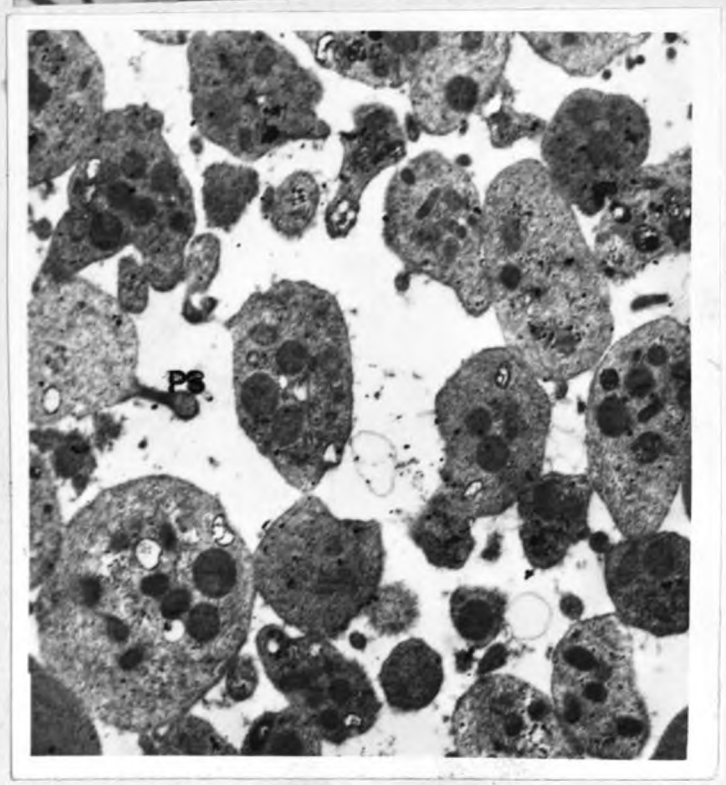
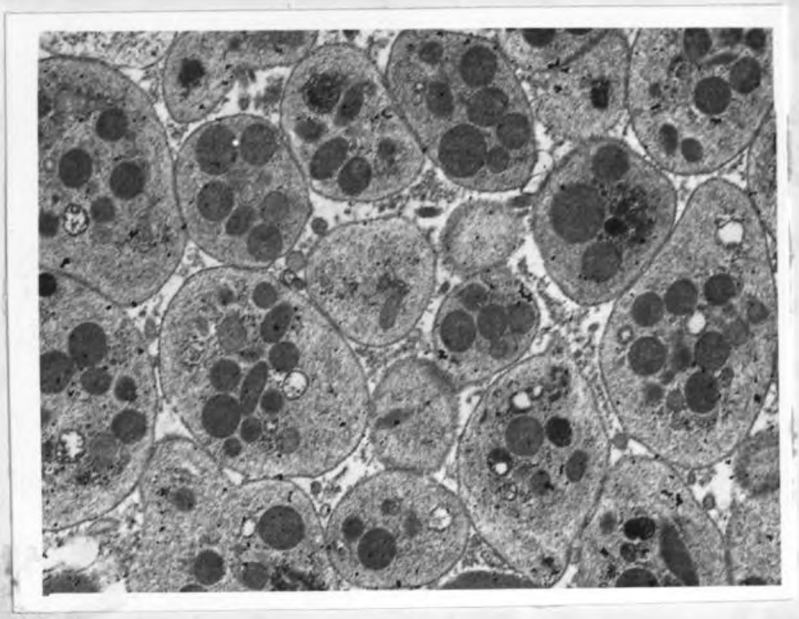

PS

Fig. 37. Electron micrograph of platelets of *T. vivax* infected steer K 121, 28 days post infection showing practically no degranulation x 13,800.



4.6.2. *Peripheral leukocytes*

Most of the mononuclear cells examined up to 7 days post infection had little cytoplasm, few mitochondria and ribosomes (Fig. 38). Their nuclei were rounded with marginated heterochromatin and large euchromatin. The granulocytes did not show any changes. On the 12th day after infection most of the mononuclear cells (Fig. 39) had large cytoplasm, abundant ribosomes, many mitochondria and cytoplasmic projections. Their nuclei were rounded with abundant marginated heterochromatin, some of which was interspersed within the euchromatin. The granulocytes showed degranulation, abundant glycogen and pseudopodia (Fig. 40). On day 13 post infection, the nuclei had distinct nucleoli, marginated heterochromatin, some interspersed within the little euchromatin. On day 18 post infection, most of the mononuclear cells had abundant and marginated heterochromatin and relatively little euchromatin. The cytoplasm was rich in ribosomes and mitochondria and had many cytoplasmic projections (Fig. 41). On day 21, some of the cells had electron dense cytoplasmic vacuoles (Fig. 42). On day 27 after infection, some mononuclear cells had segmented nuclei (Fig. 43), and the cytoplasm was rich in ribosomes and contained many mitochondria and some vacuoles.

Fig. 38. Electron micrograph of peripheral leukocytes of *T. vivax* infected steer K 123, on day 5 post infection showing little cytoplasm (C), few mitochondria (M), marginated heterochromatin and large euchromatin.

N = Nucleus.

x 13,800.

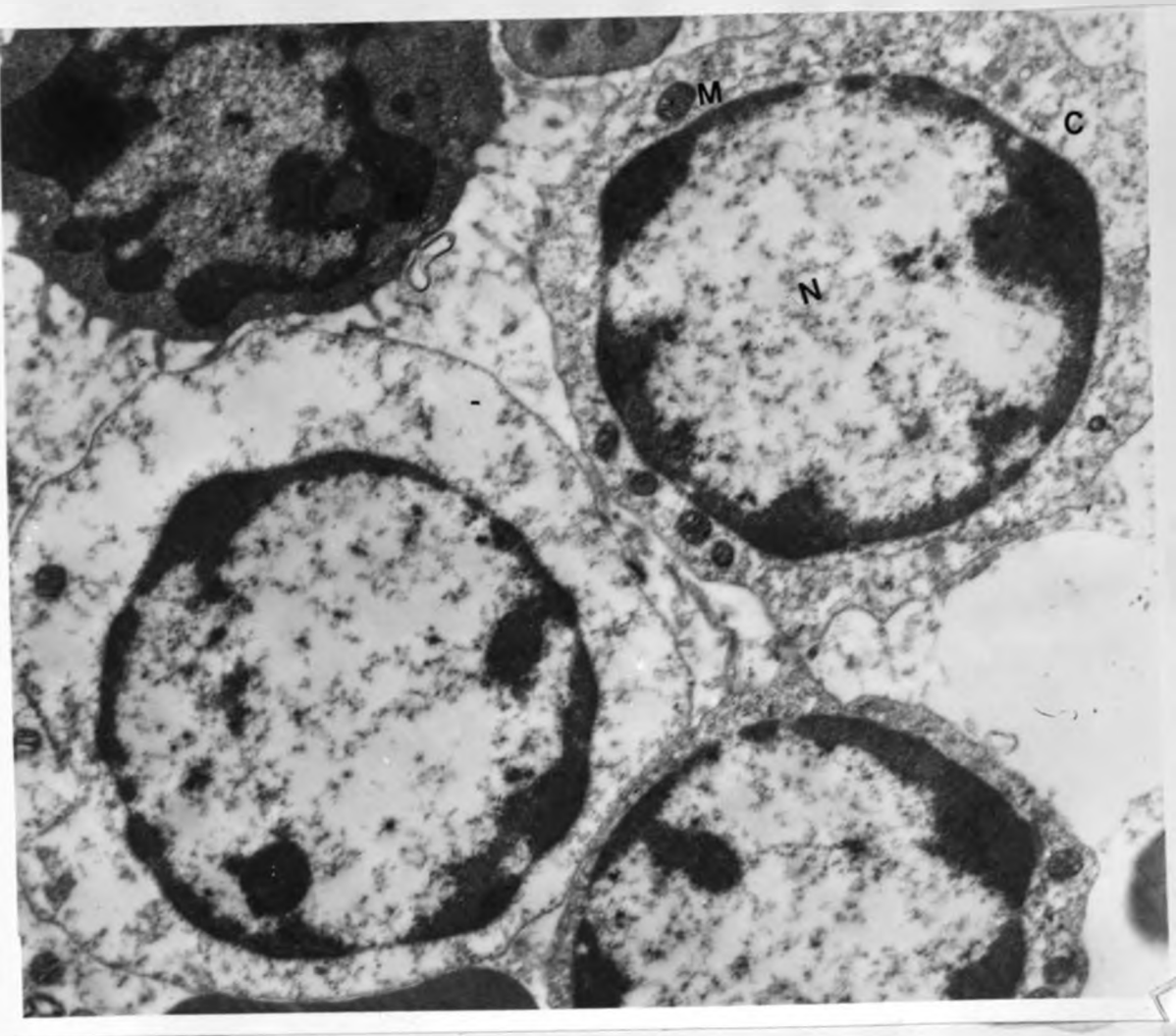

M
C
N

Fig. 39. Electron micrograph of peripheral leukocytes of *T. vivax* infected steer K 121, 12 days after infection showing slightly enlarged cytoplasm (C).

N = Nucleus, NL = Nucleolus.

M = Mitochondria, Cp = Cytoplasmic projection.

ER = Endoplasmic reticulum.

x 13,800.

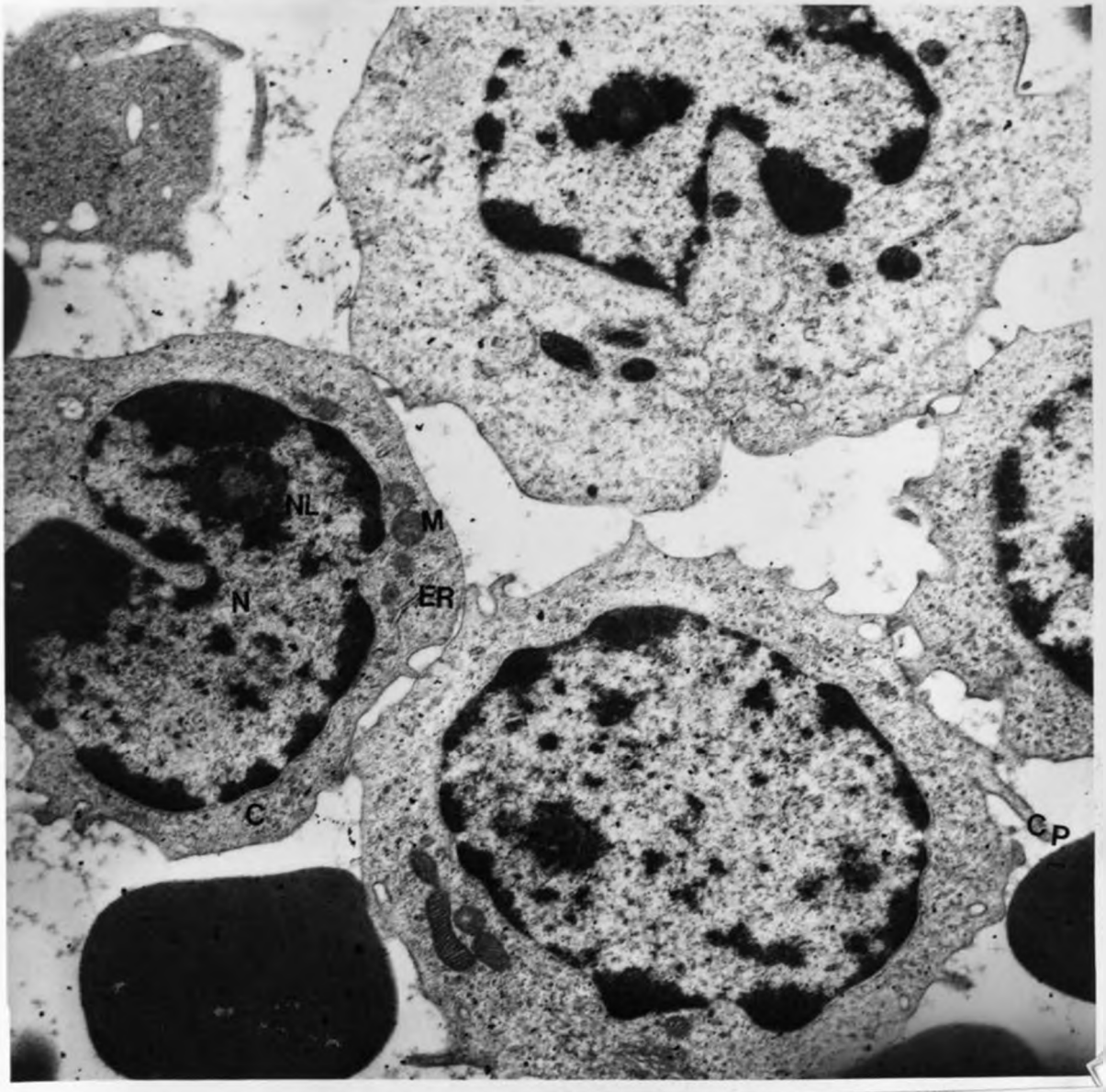

NL
M
N
ER
C
CP

Fig. 40. Electron micrograph of a peripheral leukocyte (granulocyte) of *T. vivax* infected steer K 124, 12 days post infection showing degranulation, abundant glycogen (GL) and cytoplasmic projections (Cp).

PS = Pseudopodium, N = Nucleus,

C = Cytoplasm.

x 27,000.

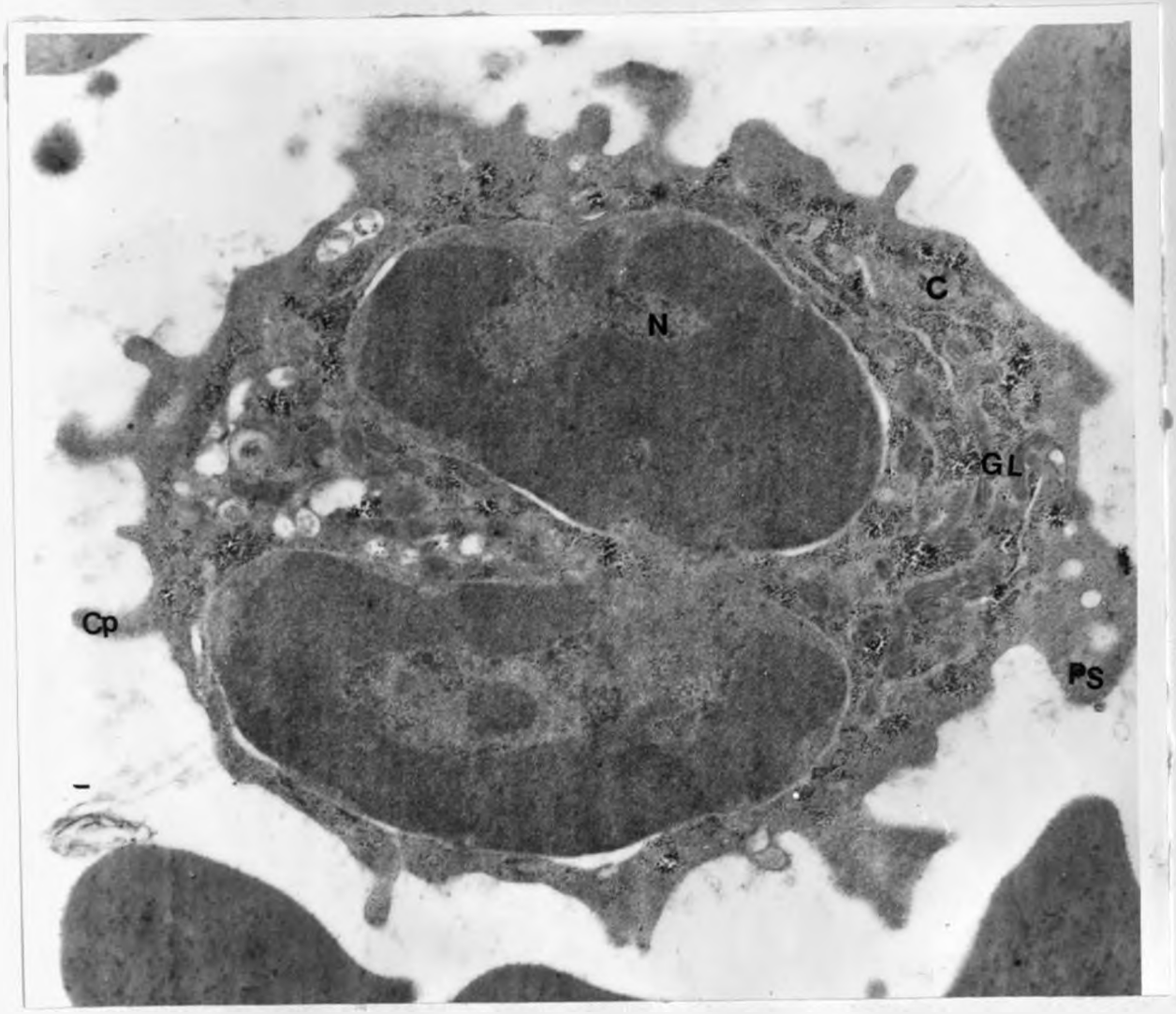

N
C
GL
Cp
PS

Fig. 41. Electron micrograph of peripheral leukocytes of *T. vivax* infected steer K 124, 18 days post infection showing large ribosome rich cytoplasm (C) with cytoplasmic projections (Cp). the heterochromatin is interspersed within euchromatin.

N = Nucleus, M = Mitochondria x 13,800.

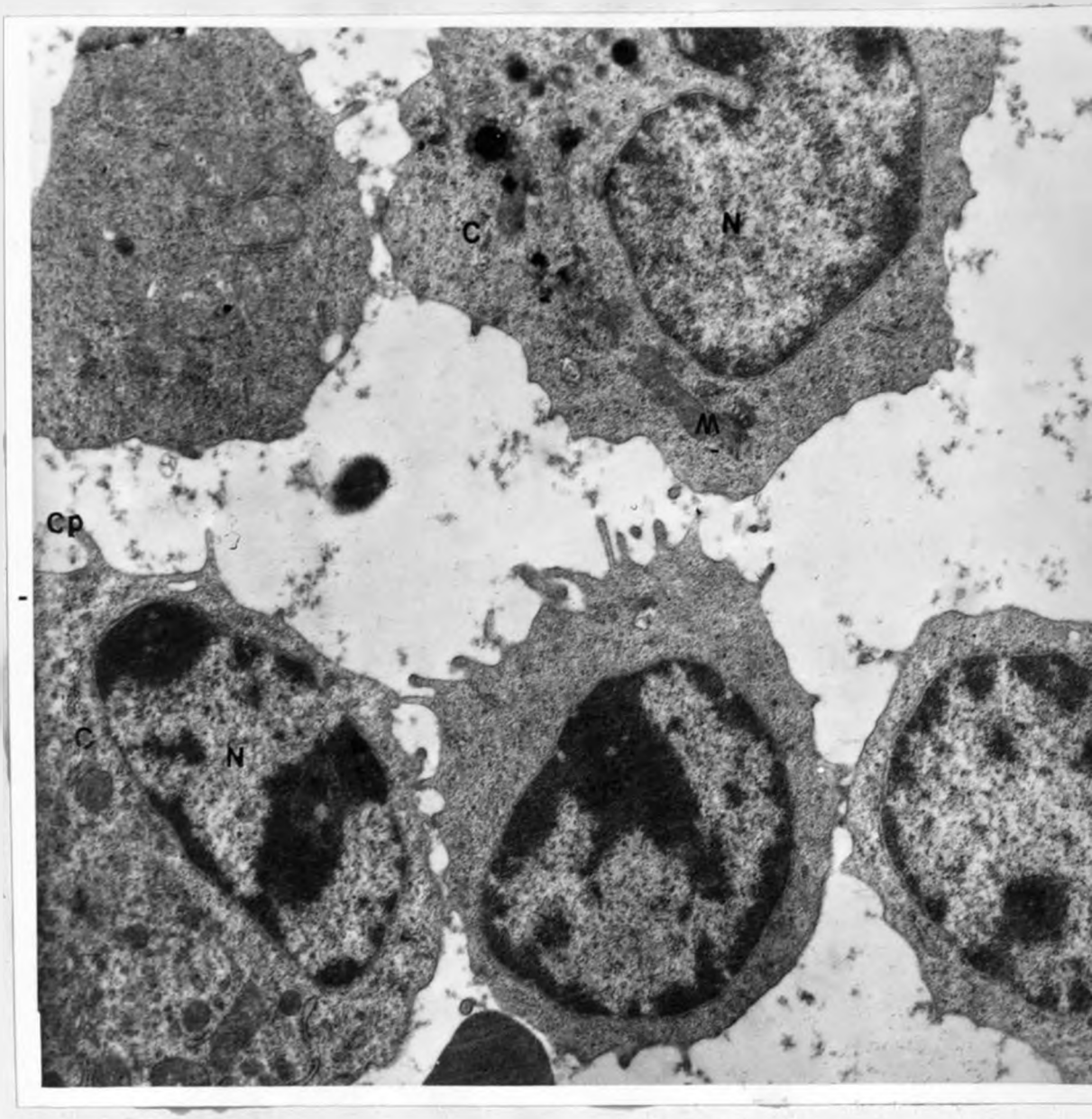
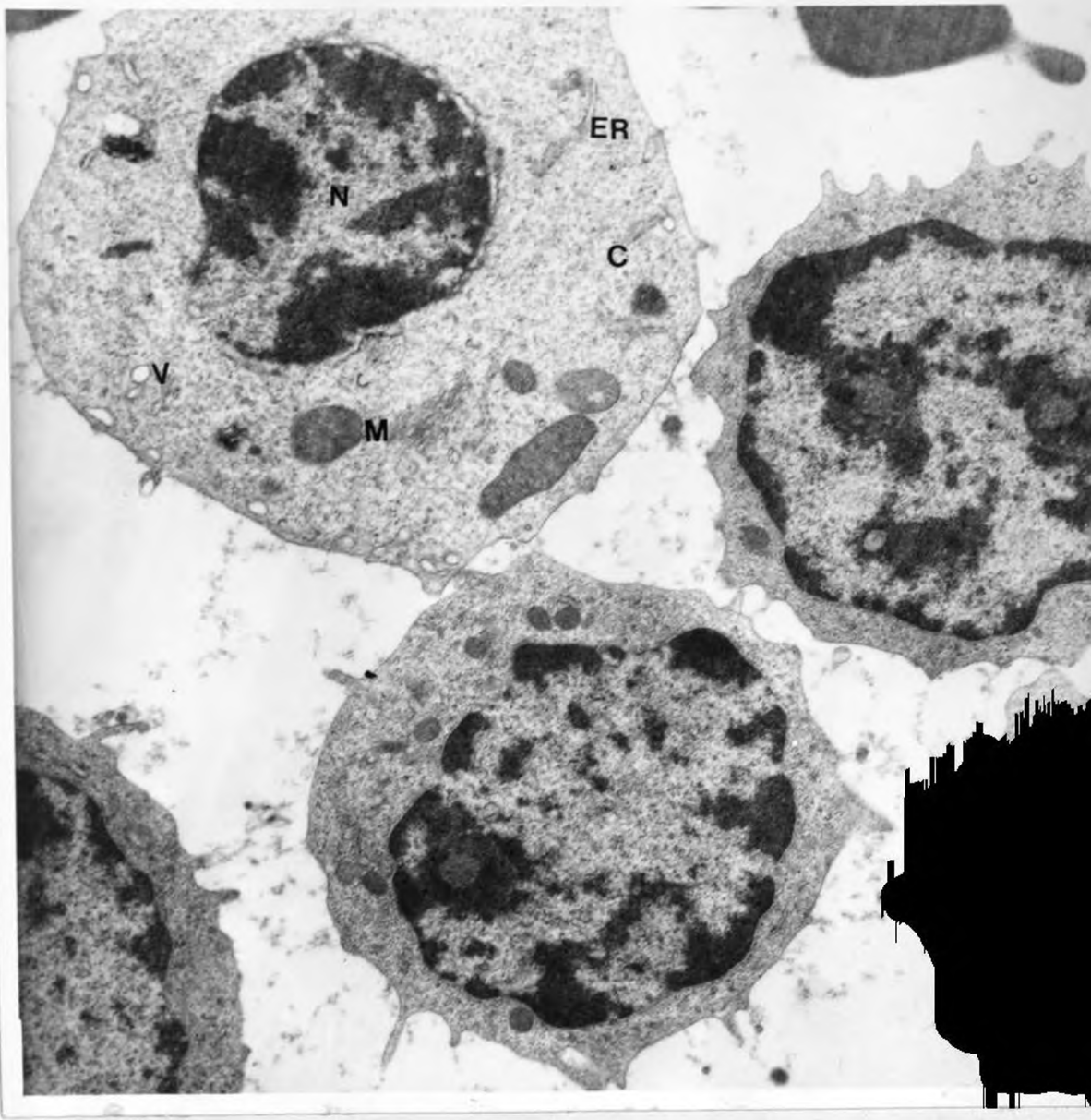

C
N
M
Cp
C
N

Fig. 42. Electron micrograph of peripheral leukocytes of *T. vivax* infected steer K 124, 21 days post infection showing enlarged ribosome rich cytoplasm (C) with vacuoles (V) and cytoplasmic projections. The nucleus (N) has abundant heterochromatin.

M = Mitochondria.

ER = Endoplasmic reticulum.

x 13,800.


ER
N
C
V
M

4.6.3. *Heart*

In the heart muscle, there was lysis and fragmentation of myofibrils and disruption of Z-bands (Fig. 44). There was also myolysis, mitochondria hyperplasia (Fig. 45). The mitochondria were of different sizes and shapes. The myocyte nucleus had abundant euchromatin and sparse heterochromatin which was also marginated and interspersed within the euchromatin. The nuclear membrane had indentations.

Haemorrhages and nuclear cells were observed in the intermyofibrillar tissue. Some of these cells were macrophages which had engulfed erythrocytes (Fig. 46). Their nuclei had indentations and abundant heterochromatin which was marginated and interspersed within the euchromatin. Their cytoplasms was rich in ribosomes and some of the cells showed pseudopodia.

The parasites were many and of various sizes and shapes but most of them were elongated (Fig. 47). They were observed between myofibres where there was oedema fluid through which they could swim. The parasite had a pellicle, nucleus, several electron dense bodies, mitochondrion, rough endoplasmic reticulum, vacuoles and free ribosomes.

Fig. 43. Electron micrograph of peripheral leukocytes of *T.* [illegible] infected steer K 121, 28 days post infection showing indented and segmented nuclei (N), ribosome rich cytoplasm (C). There is abundant heterochromatin.

M = Mitochondria.

x 13,800.

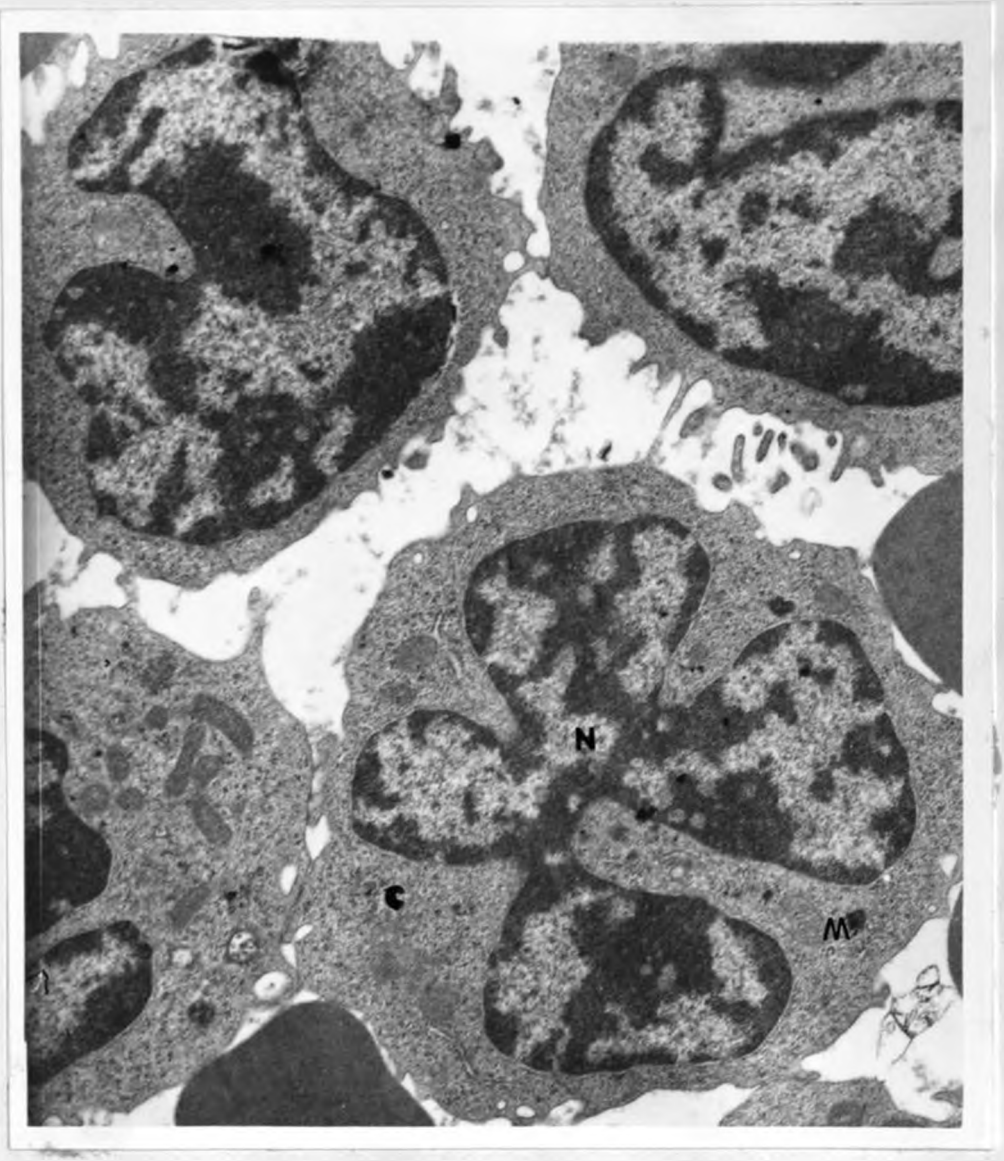

N
C
M

Fig. 44. Electron micrograph of myocardium of *T. vivax* infected steer K 122 showing lysis of myofibrils (Myl), disruption of Z-bands (Z). The parasite (P) lies between a blood vessel and a myofibre, S = Sarcoplasma, A = Actin filaments, MS = Myosin filaments. SL = Sarcolemma, SA = Sarcomere, En = Endothelial cell, x = 13,800

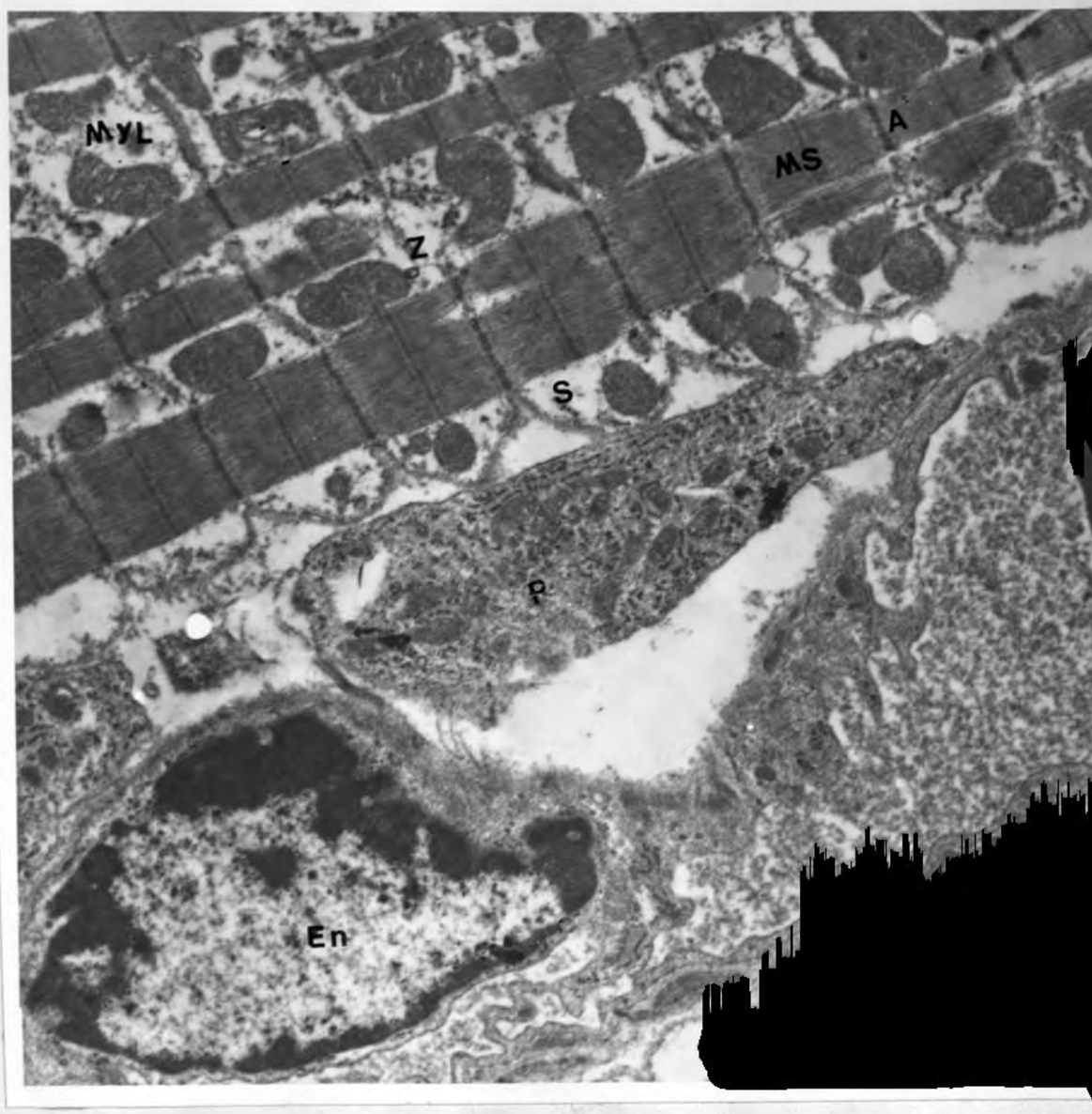

MyL
A
MS
Z
S
P
En

Fig. 45. Electron micrograph of myocardium of *T. vivax* infected steer K 122 showing lysis of myofibrils (Myl) and disruption of Z-bands (Z), N = Nucleus of a myocyte. M = Mitochondria. X 5,700.

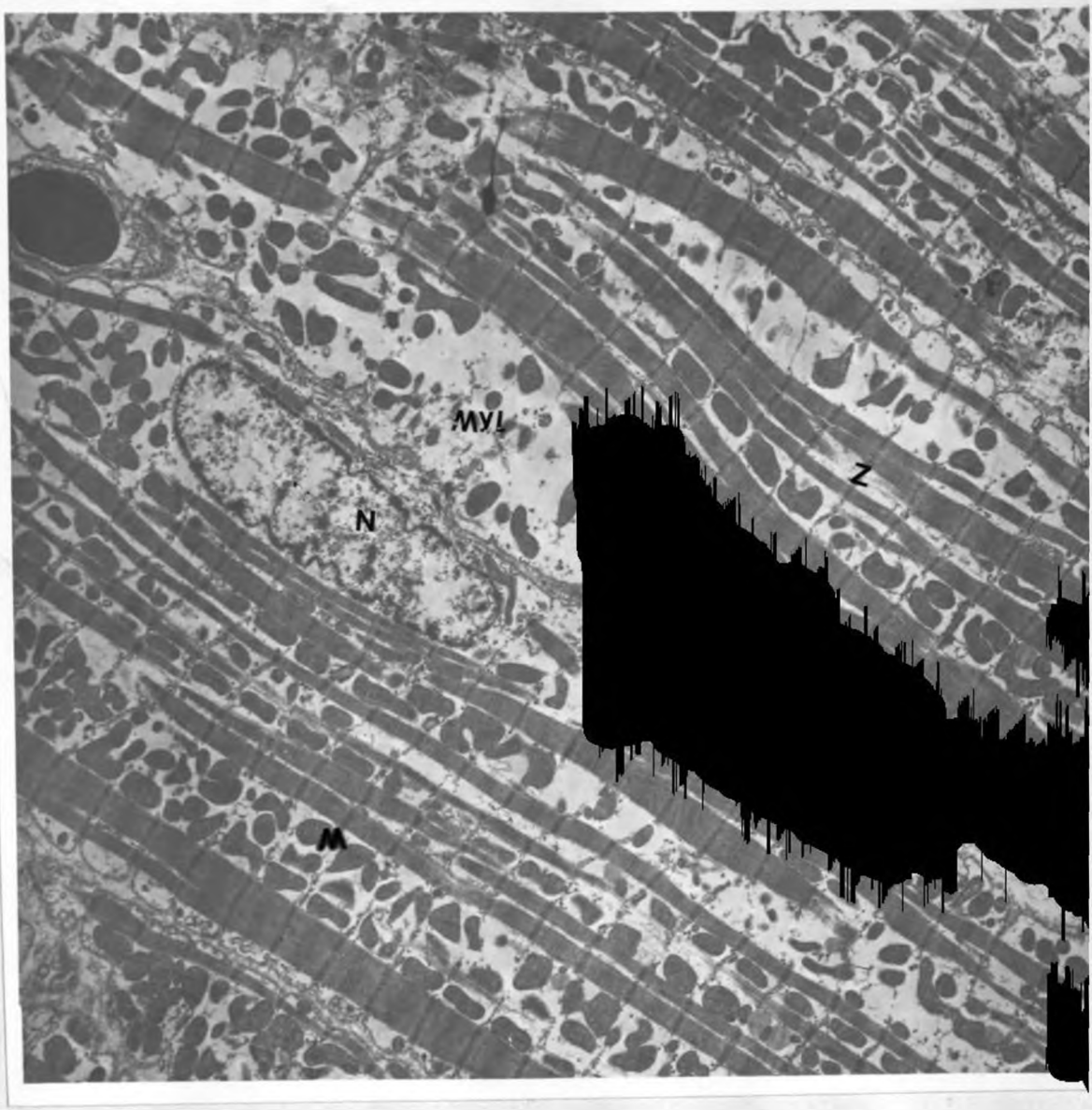

MYL
Z
N
M

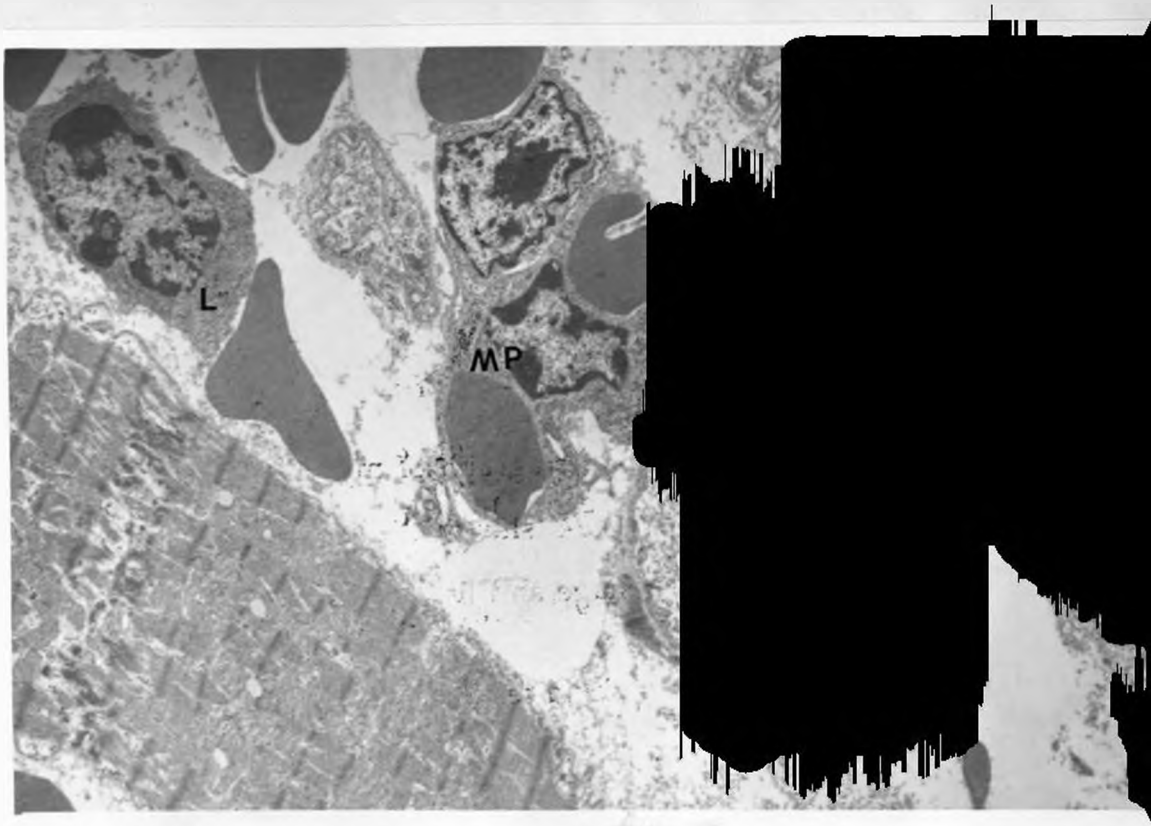
Fig. 46. Electron micrograph of myocardium of *T. vivax* infected steer K 124 showing erythrophagocytosis, interstitial haemorrhage and lymphoid cell infiltration.

L = Lymphoid cell, MP = Macrophage,

E = Erythrocyte,

MY = Myofibre

x 5,700


L
MP

4.6.4. *Blood vessels*

The blood vessels involved in haemorrhages were venules, capillaries and arterioles. The vascular walls had lost their continuity. Their lumina were reduced in size (Fig. 48). Some of the venules had collapsed (Fig. 49). Such blood vessels appeared elongated and contained some plasma but no blood cells. In the extravascular tissue of the ruptured blood vessels, there were red blood cells, inflammatory cells, plasma and in some cases parasites.

Pinocytic vacuoles were observed in the vascular wall. The nucleus of the endothelial cells showed largely marginated heterochromatin with some interspersed within euchromatin, and a distinct nucleolus. The nuclear membrane showed indentations and the cytoplasm was little (Fig. 50).

4.6.5. *Spleen*

In the spleen there were macrophages which contained red blood cells, parasites and platelets (Fig. 51). These phagocytic cells had several vacuoles, mitochondria and were rich in ribosomes. Their nuclei had sparse marginated heterochromatin and some interspersed within the large euchromatin. Lymphocytes and plasma cells were also observed and many had mitotic figures.

Fig. 47. Electron micrograph of myocardium of *T. vivax* infected steer K 123 showing a parasite (P) in oedema fluid between myofibres (MY).

M = Mitochondria,

N = Nucleus, C = Cytoplasm,

ER = Endoplasmic reticulum,

x 13,800

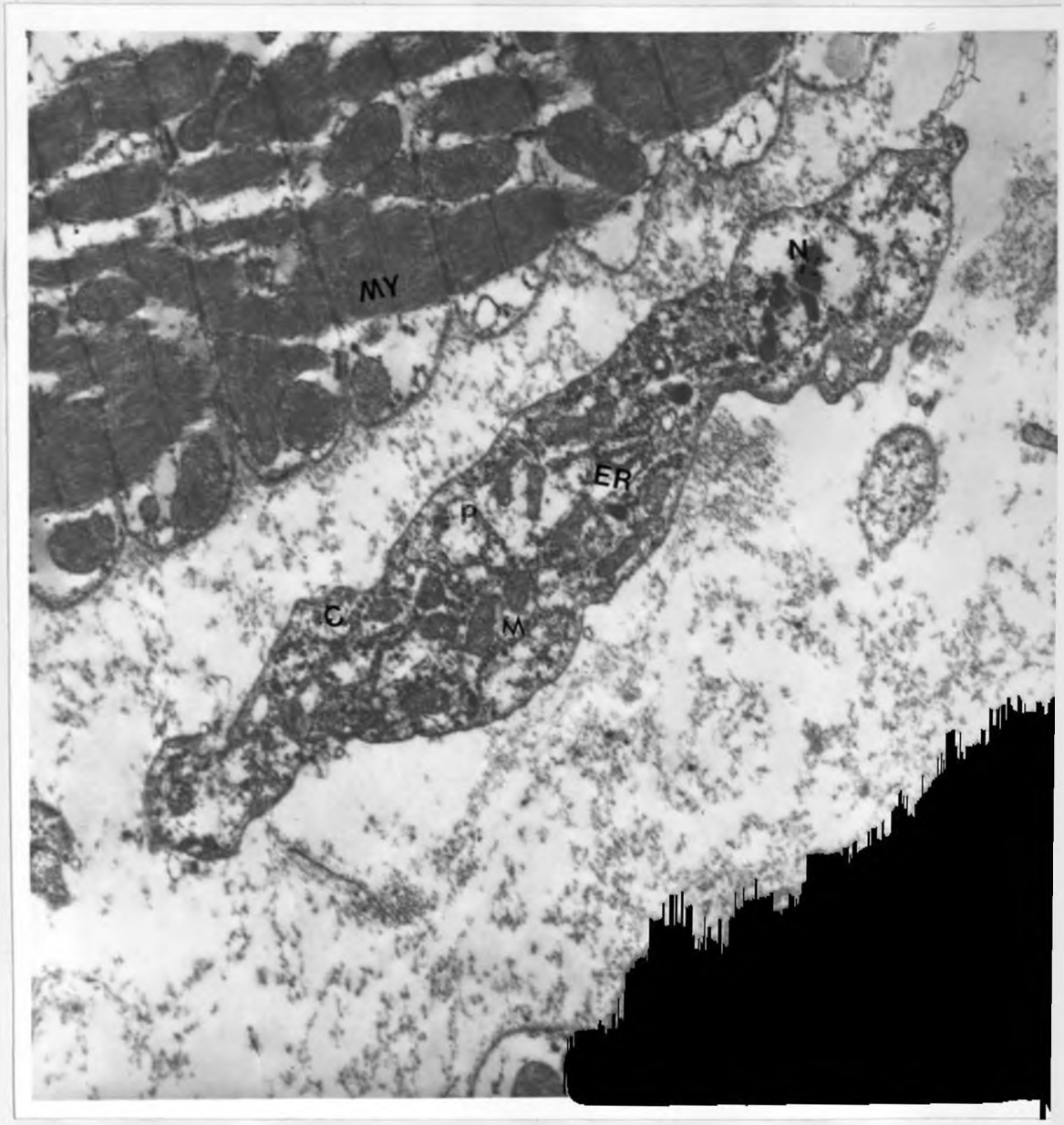

MY
N
ER
P
C
M

Fig. 48. Electron micrograph of myocardium of *T. vivax* infected steer K 123 showing a blood vessel with damaged vascular wall (arrow) and haemorrhage.

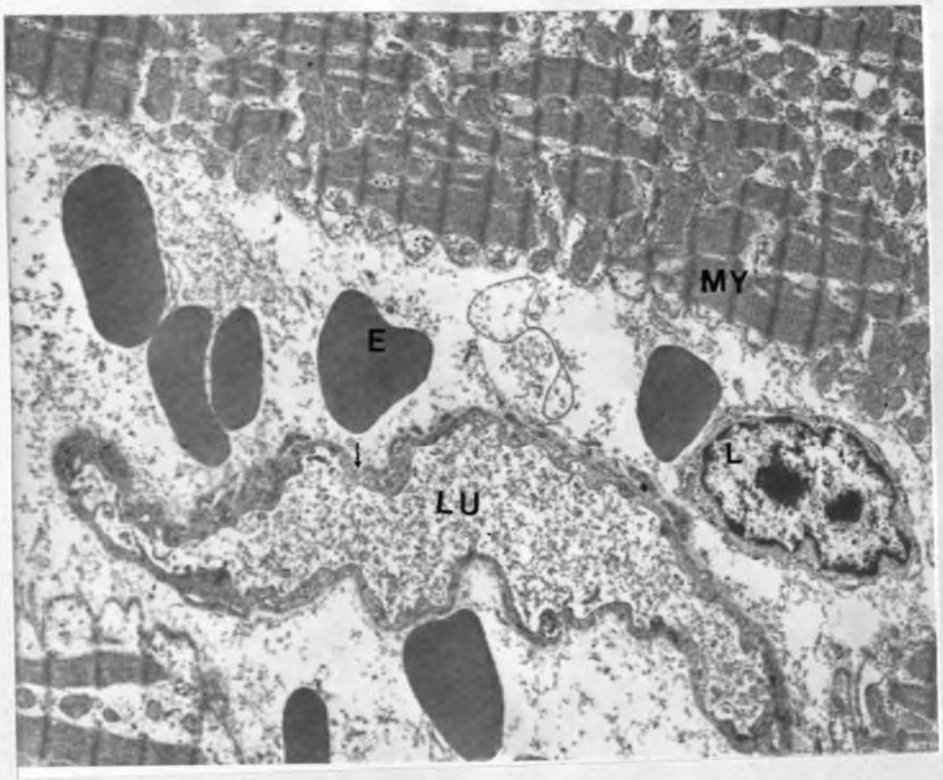
LU = Vascular lumen, E = Erythrocyte,

L = Lymphoid cell, MY = Myofibre x 7,200.

Fig. 49. Electron micrograph of myocardium of *T. vivax* infected steer K 122 showing a collapsed blood vessel with a damaged vascular wall (arrow), haemorrhage and a parasite (P).

LU = Vascular lumen, E = Erythrocyte,

RU = Rubricyte x 7,200.


MY
E
L
LU

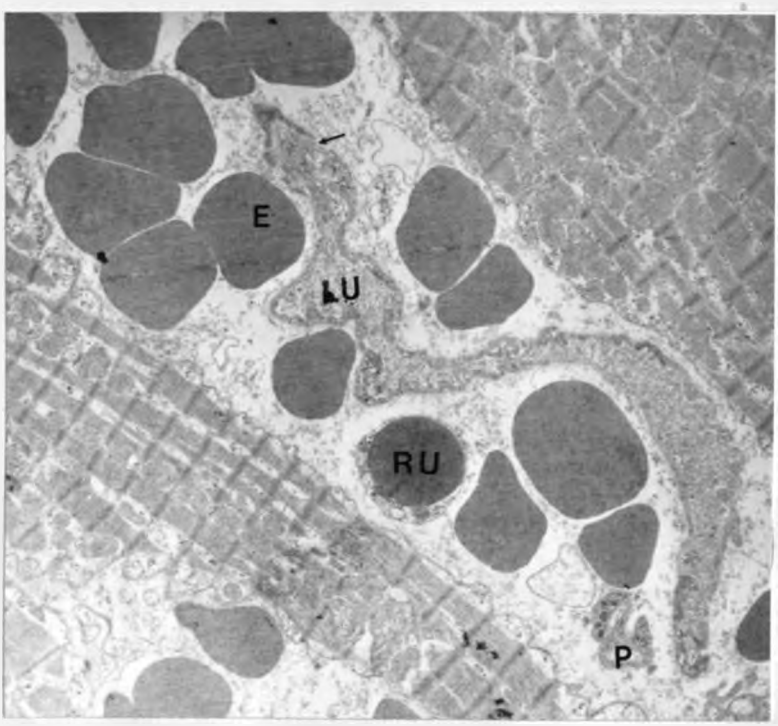

E
LU
RU
P

Fig. 50. Electron micrograph of myocardium of *T. vivax* infected steer K 122 showing blood vessel and haemorrhage.

E = Erythrocyte, L = Lymphoid cell,

N = Nucleus, NU = Nucleolus,

C = Cytoplasm, MY = Myofibre x 16,900.

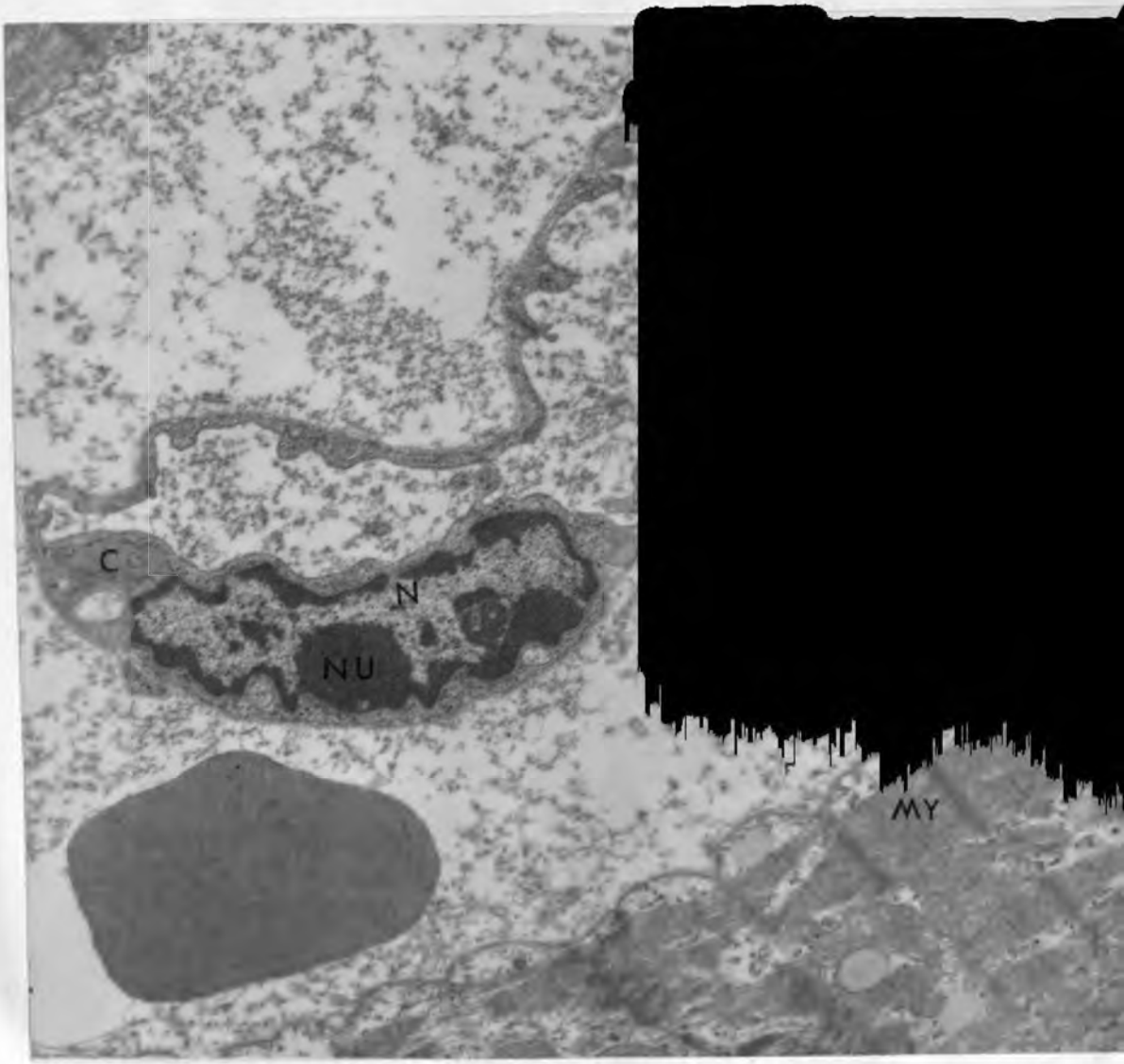
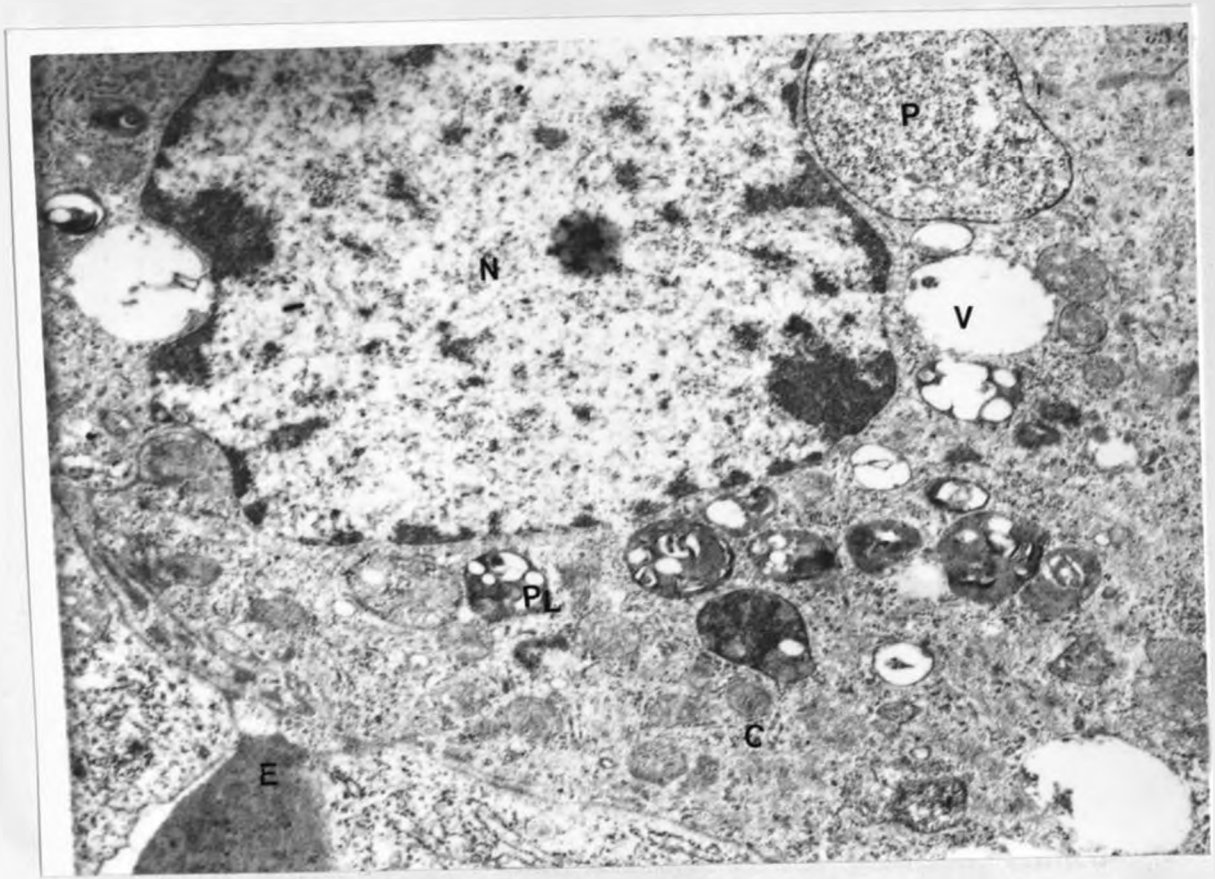

C
N
NU
MY

Fig. 51. Electron micrograph of spleen of *T. vivax* infected steer K 123 showing phagocytosis of platelets (PL), erythrocytes (E) and remnants of Parasite (P).

N = Nucleus, C = cytoplasm,

V = vacuole.

x 13,800.


P
N
V
PL
C
E

CHAPTER 5

5. Discussion

The results of the present study showed that the haemorrhagic strain of *Trypanosoma vivax* used in the experiments caused acute trypanosomiasis. The disease was characterized by pyrexia, tachycardia, tachypnoea, disseminated intravascular coagulation, tissue damage, mononuclear cell infiltration in tissues and extravascular localization of the parasites.

Fever is one of the early clinical signs of infection. It has been shown that endogenous pyrogens released following phagocytosis of parasites by neutrophils, macrophages and cells of the reticuloendothelial system (Dinarello, 1979) stimulate thermoregulatory centres leading to elevation of body temperature. The same phenomenon could have occurred in the present experimental *T. vivax* infection as fever was a constant feature. Tachycardia and tachypnoea resulted from tissue hypoxia which was caused by reduction in red blood cell count., packed cell volume and haemoglobin concentration.

Anaemia was observed clinically, pathologically and haematologically and these findings are in agreement with other reports which suggest that anaemia is the principal clinico-pathological feature of trypanosomiasis (Maxie *et al.*, 1976; Valli *et al.*, 1978; Saror, 1979; Dargie *et al.*, 1979; Facer *et al.*, 1982 and Ismail, 1988), but there is no agreement as to its pathogenesis. Three

mechanisms have been suggested, namely, haemodilution (Fiennes, 1954; Boreham, 1967; Holmes, 1976; Valli *et al.*, 1978), intravascular and extravascular haemolysis (Mamo and Holmes, 1975; Maxie *et al.*, 1978; Valli and Mills, 1980) and inhibition of erythropoiesis (Losos and Ikede, 1972 and Dargie *et al.*, 1979).

Haemolytic anaemia can be observed clinically and pathologically. Icterus and erythrophagocytosis are present in the cells of the reticuloendothelial system mainly in the liver and the spleen (Boreham, 1967; Woodruff *et al.*, 1973; Facer *et al.*, 1982). In the present study icterus and erythrophagocytosis were observed in all the infected animals though with varying intensity. These observations are in agreement with the findings of the other workers who have described haemolytic anaemia in animal trypanosomiasis (Jennings *et al.*, 1974; Murray *et al.*, 1974; Brown and Losos, 1977; Valli *et al.*, 1978). Haemolytic anaemia is caused by immunological mechanisms (Woodruff *et al.*, 1973; Murray *et al* 1974; Facer *et al.*, 1982), trypanosomal haemolysins and phospholipases (Huan *et al.*, 1975; Tizard and Holmes, 1976; Boreham, 1979). In a study on calves infected with *T. congolense*, Kobayashi *et al.* (1976) reported that erythrolysis is due to antigen-antibody complexes which are deposited on the surface of erythrocytes; and the antibody responsible is possibly IgM (Boreham, 1967). In *T. vivax* infection, immunological sensitization of erythrocytes as evidenced by a shortened life span of red blood cells (Jenkins, 1980) consists of type II or III cytotoxic reaction involving antigen-antiboby complexes (Coombs and Gell, 1975). Haemolytic anaemia can also be caused by trypanosomal haemolytic factors (Huan *et al.*, 1975; Tizard *et al.*,

1978), fever (Karle, 1974) and an active mononuclear phagocytic system (Woodruff *et al.*, 1973). Haematologically, a drop in the total red blood cell count, haemoglobin concentration, packed red blood cell volume, haemodilution and reduced life span of red blood cells are indicative of haemolytic anaemia (Valli *et al.*, 1978).

Anaemia is also caused by haemorrhages. In the present study, haemorrhages were observed and especially in the myocardium of all infected animals and in the gastrointestinal tract of two animals. Other workers (Hudson, 1944; Lewis, 1948; Mwongela *et al.*, 1981; Wellde *et al.*, 1983; Olubayo and Mugera, 1985) have also reported haemorrhages in *T vivax* infected cattle in Kenya.

Haemorrhages result from thrombocytopenia, endothelial damage and dissemination intravascular coagulation (DIC), but thrombocytopenia with or without DIC can cause haemorrhage (Davis, 1982). Thrombocytopenia is caused by platelet aggregation and platelet destruction (Davis *et al.*, 1974), shortened life span of platelets (Davis, 1982), splenic pooling of platelets (Robins-Browne *et al.*, 1975), and the effect on magakaryocytes in the bone marrow(Wellde *et al.*, 1983). Platelet aggregation is caused by antigen-antiboby complexes (Slots *et al.*, 1977; Davis, 1982), thromboxane (Haurand and Ullrich, 1985), microvascular damage either by antigen-antiboby complexes (Goodwin and Hook, 1968), trypanosomal phospholipases, proteases, free fatty acids (Tizard *et al.*, 1978) or the parasite itself as observed in the present study.

Atypical extramedullary megakaryocytes were observed in the infected animals in such organs as the spleen, lymph nodes, liver and the lungs. This is an indication that *T vivax* infection affects not only the platelets but also megakaryocytes and other cells in the bone marrow. And when the trypanosomes are killed, for example with berenil treatment, the platelets return to normal (Wellde *et al.*, 1978). Bone marrow inhibition has been reported by Losos and Ikede (1972) and Dargie *et al.* (1979). Dysthrombopoiesis causes thrombocytopenia which results in haemorrhage. As observed by Davis (1982), thrombocytopenia is a complication of African trypanosomiasis irrespective of the route of inoculation (intravenously or tsetse transmission), genus or breed of livestock species or variable antigenic types. In the present study, haemorrhagic reaction coincided with thrombocytopenia and parasitaemia. This inverse relationship between parasitaemia and thrombocytopenia has also been reported by Wellde *et al.*, (1983) and Olubayo and Mugera (1985). Dysthrombopoiesis could also result from splenomegaly, a significant pathological finding in trypanosomiasis. Splenomegaly functions antagonistically to the bone marrow resulting in dysthrombopoiesis and generally in dyshaemopoiesis.

Haemorrhages can also result from microvascular damage by antigen-antibody complexes; trypanosomal phospholipases, proteases and fatty acids, or the parasite itself. In the present study, trypanosomes were observed in the interstitium of myocardium.

The myocardial blood vessels were damaged and this led to extravasation of the parasite, haemorrhage and oedema. Oedema can also be caused by antigen-antibody complexes which release kinins, histamine and serototin resulting in increased vascular permeability as described by Boreham (1979).

The presence of fibrin monomers and fibrinogen degradation products together with thrombocytopenia and microthrombi indicates the presence of disseminated intravascular coagulation. Other workers have reported DIC in trypanosome infections based on increased fibrinogen degradation products, altered fibrinogen levels and thrombocytopenia (Barret-Connor *et al.*, 1973; Van den Ingh *et al.*, 1976; Forsberg *et al.*, 1979; Anosa and Isoun, 1983; Ismail, 1988),. DIC in *T. vivax* infection has been reported in cattle by Losos and Ikede, 1972; Van den Ingh *et al.*, (1976), Olubayo and Mugera (1985) and Ismail (1988). Greenwood and Whittle (1976) reported thrombocytopenia with DIC in patients infected with *Trypanosoma rhodesiense*. However, thrombocytopenia with or without DIC leads to haemorrhage (Davis, 1982).

In the heart, there was mononuclear cell pancarditis and the cells included lymphocytes, plasma cells and macrophages. The cellular infiltration was due to the presence of the parasite which was observed in extravascular tissue. These findings are in agreement with the findings by Urquhart (1980) who reported that myocardial cellular infiltration was due to trypanosomal antigens and immunoglobulins. The presence of infiltrating cells, haemorrhage and oedema must have exerted pressure on the my-

ofibrils resulting in their rupture and fragmentation. In some areas, there was lysis of myofibrils and relative hyperplasia of the mitochondria. Mitochondria contain lipoprotein complexes such as ribonucleic acid (RNA) and enzymes responsible for the synthesis of adenosine triphosphate (ATP) which provides energy required for various cellular processes. Apart from the numerous mitochondria present in the heart, there was also abundant glycogen which can be broken down to glucose for utilization by the myocytes. The parasite takes advantage of the abundant glucose which it utilizes for its own nutrition.

The parasites were observed extravascularly and this proves that *Trypanosoma vivax* is also a tissue parasite and cannot be regarded as strictly blood parasite as suggested by Losos and Ikede (1972) and Maxie *et al.* (1979). The present findings are in agreement with those reported by Büngener and Mehlitz (1977) and Van den Ingh *et al.* (1979), who concluded that *T. vivax* is both blood and tissue parasite.

Antigen-antibody and complement reaction release histamine (Richards, 1965) and serotonin (Slots *et al.*, 1977). These pharmacologically active substances cause vascular lesions such as increased permeability and leakage resulting in oedema as observed by Goodwin and Hook (1968) in *T. brucei* infection. Pulmonary oedema was present in infected animals in the present experiment. Pulmonary oedema has been reported by Veenendaal *et al.* (1976) in *T. vivax* infected goats. Although the parasite was not observed in the abomasal and pulmonary oedema fluids, trypanosomes are usually found in body fluids such as plasma, cerebrospinal fluid and

also in myocardial interstitial oedema fluid as observed in the present study.

Splenomegaly and erythrophagocytosis is a common feature in African trypanosomiasis (Woodruff *et al.*, 1973). The mechanism inducing erythrophagocytosis by the reticuloendothelial system is immunological sensitization of the red blood cells (Facer *et al.*, 1982), and trypanosome derived phospholipase haemolysins (Huan *et al.*, 1975; Tizard and Holmes, 1976). Following hemolysis, iron is retained in the form of hemosiderin by Kupffer cells and other cells of the reticuloendothelial system. Splenomegally functions antagonistically in the bone marrow resulting in the inhibition of medullary haemopoiesis. In splenic haemosiderosis, iron is trapped and is therefore not readily available for reutilisation in the synthesis of haemoglobin resulting in anaemia. Follicular hyperplasia of the spleen was due to proliferation of lymphoid cells and also plasma cells necessary for the production of large amounts of IgM in *T. vivax* infection (Clarkson *et al.*, 1975). Lymphoid cells were observed in small splenic blood vessels including the sinusoides and these cells metastasized to other organs such as the heart, kidney, liver and brain. This is a cellular response to the presence of trypanosome antigens. These cells also cause pressure atrophy in the affected organs such as the heart and the kidneys resulting in their impaired functions.

The presence of megakaryoctes in the spleen, lymph nodes, lungs and liver is an indication of the body trying to initiate extramedullary thrombopoiesis.

There were degenerative changes in the hepatocytes such as cloudy swelling and vacuolar degeneration especially around the central veins. This centrolobular vacuolation is due to hypoxia resulting from anaemia. Haemosiderosis was a result of erythrolysis, and the icterus present was prehepatic.

Proteinaceous material observed in the renal glomerular space could have resulted from damaged cardiac myofibres releasing myoglobin which was excreted with the urine. Other renal lesions could have been due to immune complexes as reported by Nagle *et al.* (1974) in monkeys infected with *T. rhodesiense*. The loss of proteins coupled with anorexia must have led to emaciation, which was a common feature in the present study.

Perivascular cuffing was observed in an animal which was killed 28 days after infection. This was a non-suppurative encephalitis and this is in agreement with the findings described by Mwongela *et al.* (1981) and Olubayo and Mugera (1987) in *T. vivax* infected cattle.

The ultrastructural changes in peripheral leukocytes were similar to those observed in mononuclear cells infiltrating various organs. It was observed that in the course of the disease,the cytoplasm of these cells enlarged with abundant free ribosomes, mitochondria and rough endoplasmic reticulum. Mitotic figures, pinocytosis, phagocytosis, nuclear segmentation and cytoplasmic projections were common and are an indication of increased cellular activity, multiplication and protein synthesis in response to parasite antigens.

The present study has shown that emaciation, anaemia, leukopenia, thrombocytopenia, haemorrhages and mononuclear cell infiltration are common features in cattle infected with a haemorrhagic strain of *T. vivax*. Haemorrhages were caused by thrombocytopenia, DIC and microvascular damage. Thrombocytopenia resulted from platelet destruction, platelet consumption during DIC and from dysthrombopoiesis. Another common lesion was haemorrhagic pancarditis with fragmentation of the myofibres, and the presence of trypanosomes extravascularly. Thrombocytopenia was always inversely proportional to parasitaemia.

CHAPTER 6

6. Conclusion.

A study was undertaken to determine the clino-pathological changes, ultrastructural changes in tissues, peripheral leukocytes and platelets, haematological parameters, levels of parasitaemia, thrombocyte counts, megakaryocyte changes, total plasma protein concentration, plasma fibrinogen concentration and protamine sulphate paracoagulation test for fibrin monomers and fibrinogen degradation products in cattle experimentally infected with *T. vivax* (KETRI 2445).

Out of the 10 infected animals, 2 died 22 days after infection. They had severe haemorrhagic myocarditis, gastroenteritis, pulmonary oedema and nephritis. The other animals were killed in extremis and they also showed similar but less severe lesions. All the animals had anaemia, leukopenia and thrombocytopenia which was inversely proportional to parasitaemia.

Haemorrhages were caused by thrombocytopenia, disseminated intravascular coagulation and microvascular damage. Platelet destruction, dysthrombopoiesis and platelet consumption as part of DIC were the cause of thrombocytopenia. Microvascular damage as observed in the heart was caused by the parasite. In several organs, there was mononuclear cell infiltration and this was a response to the parasite antigens and antigen-antibody complexes.

Myocardium showed fragmentation of the myofibres and Purkinje cells and this resulted in cardiac malfunction. Parasites were localized extravascularly confirming that *T. vivax* is both a plasma and tissue parasite.

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