THE ROLE OF KUPFFER CELL SURFACE LECTIN IN ERYTHROPHAGOCYTOSIS IN BOVINE *Trypanosoma vivax* INFECTION

BY

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BY

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DECLARATION

I do declare that this work was carried out by me and has never been published elsewhere. It was carried out in the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, NIGERIA.

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CERTIFICATION

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ABSTRACT

Desialylated erythrocytes from experimental *Trypanosoma vivax* infected goats and cattle were interacted with their respective homologous isolated liver kupffer cells in the presence of bovine calf serum, with and without lactose. The highest percentage of desialylated erythrocytes bound to Kupffer cells in conjunction with erythrophagocytosis was between 12 and 19% and 13 and 14% for the infected goats and cattle respectively. But, summing the latter percent values with regards to Kupffer cells bound to desialylated erythrocytes without erythrophagocytosis, and the existence of erythrophagocytosis without binding of red cells, much higher values of 71% and 62% were obtained in the *T. vivax* infected goats and cattle, respectively, without trace of lactose.

Interactions of the desialylated erythrocytes from *T. vivax* infected goats in the presence of lactose, or *T. vivax* infected cattle intravenously infused with 0.5 g per kg body weight lactose, both in the presence of bovine calf serum, showed remarkable inhibition of both binding and erythrophagocytosis. Low values of *2 %* and *0-1 %* were obtained in goats and cattle, respctively. The inhibitory effect was accomplished through competitive binding of the kupffer cells surface lectin by lactose. The results suggest strongly that the erythrophagocytosis in acute ruminant *T.vivax* anaemia is partly lectin based, and the significance of these findings were discussed in relation to the control of trypanosomiasis.

In a separate experiment, 2 out of 4 *T. vivax* infected, and 2 out of another 4 *T. vivax* infected-lactose infused Sokoto Gudali calves, including one out of 4 control calves were sacrificed for pathological examinations. Except for the 4 control, the remaining 8 calves were experimentally infected using 11 x 10^6 *T. vivax* trypanosomes, intravenously, as a source of neuraminidase. At the peak of parasitaemia, lactose was intravenously infused at the rate of 0.5 g kg^{-1_1} body weight into 4 of the infected calves, to inhibit the binding of desialylated erythrocytes to kupffer cells

(macrophages), and therefore, subsequent erythrophagocytosis. This resulted in amelioration of the anaemia in the 4 infected, lactose infused calves. However, the other 4 infected, non-lactose infused calves had marked anaemia. In contrast, the 4 infected, lactose infused calves shoved no gross—lesions except for mild anaemia and emaciation.

The major gross lesions observed in the infected, non-lactose infused calves included rough hair coat, emaciated carcases, serous atrophy of coronary fat, enlarged oedematous lymph nodes, and hepato—splenomegaly. Hovever, these changes were not observed in the infected, lactose infused calves and the non-infected control calf.

Histopathologically, the 2 infected, lactose infused calves had narrow hepatic sinusoidal space, enlarged Bowman's space, hypercellular renal glomeruli, and a less dense lymphoid hyperplasia (proliferation of lymphocytes) in the spleen and lymph nodes compared to the infected, non-lactose infused calves. In addition, no significant histopathological changes occured in the heart, lung, and intestine of the infected, lactose infused calves. However, in the infected, non-lactose infused calves there were diffuse hepatic necrosis and erythrophagia by Kupffer cells, dense lymphoid hyperplasia(active lymphoid follicles with plasma cells), and haemosiderosis. There were haemosiderin laden macrophages in the speen, liver, kidney, and lymph node. Hypercellular glomeruli, necrosis of the proximal, distal and collecting tubules of the kidney with periglomerular and inter-tubular mononuclear cellular infiltration. There was minimal myocarditis, while the brain, lung and the intestine of the calves had no significant histopathological lesions.

No significant microscopic lesions were observed in the liver, kidney, lung, heart, spleen, lymph node, brain, and the intestine of the control calf examined.

The results of this study have shown that lactose plays a vital role in the prevention or amelioration of tissue injury and cellular reactions in various organs of the body during the course of bovine trypamosomiasis. This finding further supports the safety use of lactose without causing any damage to body organs. As such, its incoporation vith other chemotherapeutic agents for treatment of trypanosomiasis is, therefore, strongly recommended.

In a third experiment using abbatoir samples, the interaction between neuraminidase treated bovine erythrocytes and homologous kupffer cells in the absence of lactose (an inhibitor) resulted in a very high erythrocyte binding and erythrophagocytosis (58-93%). However, binding and erythrophagocytosis fell to between 3 and 16% following interaction of the desialylated erythrocytes with homologous Kupffer cells in the presence of lactose.

Still using abbatoir samples, interactions between neuraminidase untreated erythrocytes and homologous Kupffer cells in the absence of lactose resulted in 12 to 65% binding and erythrophagocytosis rate. However, interaction between such untreated erythrocytes and homologous Kupffer cells in the presence of lactose yielded the least observed binding and erythrophagocytosis rate of 0-8%.

The decreased binding and subsequent erythrophagocytosis was due to the inhibitory effect of lactose on both processes. The high level of binding and erythrophagocytosis (12-65%) observed following interaction between untreated erythrocytes and homologous Kupffer cells in the absence of lactose suggested that some of the slaughtered abbatoir cattle were carrying subclinical infections due to sialic acid cleaving organisms, such as trypanosomes.

DEDICATION

This work is entirely dedicated to my parents for their unforgettable efforts of bringing me up to this great stage. May Allah reward them abundantly and may the blessings of Allah be with them, Ameen.

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ABBREVIATION, DEFINITIONS, GLOSSARIES SYMBOLS:

% = Percent
mg = Milligram
ml = Millilitre
g = Grram
mU = Millimicron
β = Beta

CHAPTER 1

1.1 GENERAL INTRODUCTION

Trypanosomiasis is a very serious disease of man and his domesticated animals in many parts of the tropics and sub-tropics. In Africa, trypanosomiasis assumes a continental dimension since the trypanosomes are cyclically transmitted by *Glossina* species (tsetse flies), which infest about 10 million square kilometers south of the Sahara. In other parts of the world, transmission is mechanical by blood sucking arthropods (Connor, 1991). The most important of animal trypanosomes are those belonging to the monomorphic group, notably, *Trypanosoma congolense* and *T.vivax*. Both trypanosomes are widely distributed and are responsible for great economic losses in the livestock industry (Fiennes, 1970). However, the *brucei* group of polymorphic trypanosomes is also of great medical importance since it includes the human trypanosomes *T.rhodensiense* and *T.gambiense*. Another member of this group, *T.brucei*, infects wild and domestic ungulates causing subclinical or clinical disease (Fiennes, 1970).

Despite the fact that *T.vivax* infection has for a long time been recognized as a major cause of poor production and losses in cattle in West Africa (Edward *et al.*, 1956), the pathogenesis and pathology of bovine trypanosomiasis has remained the subject of intensive investigation (Saror, 1980).

A principal pathological feature of trypanosomiasis is anaemia, and experimental evidences have indicated that the anaemia is haemolytic in nature. The haemolysis occurs principally extravascularly in an expanded mononuclear phagocytic system (Fiennes, 1970; Naylor, 1971; Losos and Ikede, 1972; Van Den Ingh *et al.*, 1976).

Reduction in the erythrocytes life-span and erythrophagocytosis are consistent contributory factors in the aetiology of the anaemia of bovine trypanosomiasis (Mackenzie and Cruickshank, 1973; Valli *et al.*, 1978; Valli

and Forsberg, 1979; Dargie *et al.*, 1979b). Significant cleavage of erythrocyte surface sialic acid during peak parasitaemias and, thence, highest production of trypanosome sialidase (Esievo, 1979) were incriminated in the shortening of erythrocyte life-span and erythrophagocytosis in bovine trypanosomiasis (Esievo *et al.*, 1982). Much support for this was found in the numerous quantitative and qualitative biochemical differences between the erythrocyte sialic acid of the trypanosusceptible Zebu and the trypanotolerant N'dama cattle, which also provided a favourable explanation to the N'dama's trypanotolerance (Esievo *et al.*, 1986; 1990; Shugaba *et al.*, 1994). These differences include the presence of an additional band (or sialic acid) on the surface of the N'dama erythrocyte which is absent in the Zebu (Esievo *et al.*, 1990). Higher concentrations of erythrocyte surface sialic acids, O-acetyl, but less glycolyl groups occur in N'dama cattle while the reverse is the case in the Zebu (Shugaba *et al.*, 1994).

Esievo *et al.* (1982) postulated that, at peak parasitaemia, trypanosome sialidase significantly cleaves off erythrocyte surface sialic acids causing physico-chemical damage on the desialylated erythrocyte rendering it more prone to premature phagocytosis and destruction by the reticuloendothelial system. It has been shown that these physico-chemical damages are in fact due to galactose residues exposed on the erythrocyte membrane in the *T. vivax* infected cattle. This was confirmed by the binding and rosetting of such desialylated erythrocytes by freshly isolated rat peritoneal macrophages (Omage *et al.*, 1996; personal communication). Similar erythrocyte galactose exposure was achieved with commercially-produced bacterial sialidase (Jancik *et al.*, 1978). The present study was aimed at determining the roles of lectin and lactose in the binding and subsequent erythrophagocytosis of desialylated erythrocytes by Kupffer cells in acute bovine trypanosomiasis.

The importance of *T. vivax* infection in domestic animals, especially cattle, in Nigeria and other tropical African countries necessitated the present investigation on the disease with the following objectives:

i) To investigate the role of lectin on Kupffer cells membrane in erythrophagocytosis in relation to the pathogenesis of the anaemia in acute bovine trypanosomiasis.

ii) To investigate the role of lactose (an inhibitor) in the inhibition of binding, hence erythrophagocytosis in acute bovine trypanosomiasis.

iii) To examine the role of lactose in the maintenance of the cellular morphology and/or architecture in the various tissues and organs of the body and its possible ability to prevent cellular and tissue injury in acute bovine trypanosomiasis.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Trypanosomiasis

Trypanosomiasis (trypanosomosis) is a very important protozoal disease of animals and humans in Nigeria and other parts of the world. The disease is caused by protozoan parasites belonging to the genus *Trypanosoma*. Typically, trypanosomes are transmitted from infected animals or humans to other susceptible animals or humans by insect vectors belonging to the genus *Glossina*, commonly known as tsetse flies. Some trypanosomes (*T.vivax*, *T.evansi*, and *T.cruzi*) may be transmitted mechanically by biting flies. Haematophagous insects like Haematophota, Crysops, Stomoxys, and Hippobosca are the major biting flies affecting animals, while in humans, the Triatomic bugs are involved with the transmission of Chaga's disease caused by *T.cruzi*.

Trypanosomes are unicellular flagellate protozoan parasites belonging to the family Trypanosomatidae. The family has many genera and species which are transmissible to vertebrate hosts through insect vectors. Trypanosomes can be categorized into two major groups; (a) Salivarian, and (b) Stercorarian (Soulsby, 1968; Adam *et al.*, 1971).

2.2 The Salivarian Trypanosomes

The trypanosomes in this group are grouped into three subgenera based on differences in their morphology and pattern of development within the tsetse fly (Soulsby, 1968; Adam *et al.*, 1971). These subgenera are *Trypanozoon*, *Nannomonas*, and *Duttonella*.

These trypanosomes are found in tropical Africa within the latitudes 14°N and 30°S where *Glossina* species serve as vectors.

2.2.1 Subgenus Trypanozoon (brucei group)

The characteristic morphological features of these haemoparasites include a slender shape, tapered ends, a long free-flagellum, an undulating membrane with conspicuous wavy flagellum projecting from the surface of the trypanosome, and a small subterminal kinetoplast. *T.b.brucei*, *T.b.gambiense*, and *T.b.rhodensiense* belong to this group. They are restricted to tropical Africa and are transmitted cyclically by tsetse flies. The final developmental phase occurs in the salivary glands of the fly. The trypanosomes are pleomorphic in the blood of the vertebrate host.

T.evansi and *T.equiperdum* occur outside tropical Africa and are respectively transmitted mechanically by blood sucking flies and through copulation. However, *T.evansi* and *T.equinum* are monomorphic in the blood (Soulsby, 1968; Adam *et al.*, 1971)

2.2.1.1 Development of *T.brucei*, *T.rhodensiense* and *T.gambiense*

These trypanosomes multiply by binary fission in fluids. They exist in three forms in the blood. (1) The *slender forms* with the characteristic features of the subgenus trypanozoon; (2) the *intermediate forms* which are broader than the slender forms, having a blunt posterior end, short free flagellum, and a nucleus that may be displaced posteriorly; and (3) the *stumpy forms* are short and broad with blunt posterior end and have no free flagellum. All these three forms have conspicuous undulating membrane (Adam *et al.*, 1971).

2.2.1.2 *T.b.brucei*

Animals affected by this trypanosome include horses, donkeys, mules, camels, cattle, sheep, goats, pigs, dogs, and various wild ungulaltes. The major vectors of this trypanosome include *G.morsitans*, *G.tachinoides*, and *G.palpalis*.

T.brucei causes Nagana in domestic animals. The infection is most severe and usually lethal in equidae, camels, and dogs. However, it is mild in other domestic animals.

2.2.1.3 *T.b.rhodensiense*

This trypanosome affects humans and is cyclically transmitted by *G.morsitans*, *G.swynnertoni*, and *G.pallidipes*.

The disease caused by *T.rhodensiense* is known as sleeping sickness in humans. The disease is acute, mainly restricted to East and Southern Africa, and can be fatal unless treated. Man gets infected as a result of a bite by tsetse flies carrying infective trypanosome(metacyclic form). This usually happens after taking a bloodmeal from an infected wild game, domestic animal, or man.

2.2.1.4 *T.b.gambiense*

This trypanosome affects humans and domestic pigs. It is transmitted cyclically by *G.palpalis* and *G.tachinoides*. It is known to be the cause of chronic sleeping sickness of man in West Africa and in the region of Lake Victoria. There is a gradual development of the disease, persisting for 1 or 2 years before death. Epidemics occur when humans are bitten by tsetse flies carrying infective trypanosomes.

2.2.1.5 *T.b.evansi*

The animals affected by *T.evansi* include horses, donkeys, mules, camels, cattle, buffaloes, sheep, goats, pigs, dogs, and various wild animals.

The main vectors are haematophagous insects that transmit the infection mechanically. The disease is known commonly as *Surra* in North Africa,

Southern Asia, Indonesia, Central and South America, and the Phillippines. It is more pathogenic in horses, donkeys, mules, camels, and dogs.

2.2.1.6 *T.b.equinum*

T.b.equinum affects horses, donkeys, mules, camels, cattle buffaloes, sheep, goats, pigs and dogs. It is mechanically transmitted by haematophagous insects. The disease is important in North and Central Africa. The trpanosome lacks a kinetoplast .

2.2.1.7 *T.b.equiperdum*

This trypanosome affects specifically horses and donkeys. It is the only trypanosome that is sexually transmitted through coitus.

The disease is commonly known as *Dourine*. During the acute stage of the disease, the trypanosomes are localized in the prepuce. The parasites are rarely found in the blood (Soulsby, 1968; Adam *et al*, 1971).

2.2.2 Subgenus Nannomonas (congolense group)

Trypanosomes belonging to this subgenus are transmitted cyclically by *Glossina* species and they include *T.congolense, T.simiae, and T.godfreyi* (Mohammed, 1991).

2.2.2.1 *T.congolense*

T.congolense infects horses, camels, cattle, sheep, goats, pigs, and various wild ungulates. The major vectors include *G.morsitans morsitans, G.palpalis, G.longipalpis, G.morsitans submorsitans,* and *G.austeni*.

The disease occurs exclusively in tropical Africa. *T.congolense* is a small trypanosome with a blunt posterior end, poorly developed undulating

membrane, lacks a free flagellum, and is tapered towards the anterior end. The kinetoplast is at one side and lies near the posterior end. Its small size, lack of free flagellum, and marginal position of the kinetoplast are the most important morphological characteristics of *T.congolense*.

The trypanosomes are variable in length. The longer forms have tapered posterior end. They can be detected easily in the blood at the acute stage of the disease. Later they become scanty or absent from the peripheral blood when the infection is getting into the chronic stage. Some strains of *T.congolense* establish readily in the laboratory rodents while others seem to be refractory.
Animal inoculation using infected blood may assist in diagnosis (Soulsby, 1968; Adam *et al.*, 1971).

2.2.2.2 *T.simiae*

T.simiae affects pigs, camels, and wild suids. The major vectors are *G.morsitans* and *G.brevipalpalis*. The infection in domestic pigs results in a fulminating and rapidly fatal disease. Mechanical transmission by haematophagous insects plays a very important role in the spread of the disease.

T.simiae is pleomorphic in the peripheral blood and is morphologically similar to *T.congolense*. However, long forms with tapered posterior ends, prominent undulating membranes, and free flagella exist (Soulsby, 1968; Adam *et al.*, 1971).

2.2.3 Subgenus Duttonella (vivax group)

Members belonging to this subgenus are also cyclically transmitted with the cyclical development within the tsetse entirely restricted within the proboscis. Those trypanosomes that pass into the midgut with the bloodmeal do not survive, while those that adhere to the walls of the labrum change

into epimastigotes and multiply. As their number increases they move towards the tip of the proboscis and invade the hypopharynx where the infective metacyclics develop.

2.2.3.1 *T.vivax*

All domestic animals except dogs and various wild animals can be affected. The vectors include *G.morsitans* and *G.techinodes*.
T.vivax occurs in tropical Africa within the tsetse fly infested zones where it is transmitted cyclically. It occurs also in Central and Southern Africa and the West Indian Islands where it is transmitted mechanically especially by tabanid flies. Various strains of *T.vivax* that differ in virulence are known to exist. The disease is more severe in cattle, while pigs are most resistant (Soulsby, 1968; Adam *et al.*, 1971). *T.vivax* can be distinguished from other salivarian trypanosomes by its rapid movement in peripheral blood. This accounts for the name *vivax* meaning lively (Adam *et al.*, 1971).

The characteristic features of *T.vivax* are the rounded posterior end, a relatively large kinetoplast which lies at or very near to the posterior end, a long free flagellum, a prominant undulating membrane, and a broad shape. It is not infective to laboratory rodents (Soulsby, 1968; Adam *et al.*, 1971).

2.3 The Stercorarian Trypanosomes

These trypanosomes are characteristically host specific and non-pathogenic except in the case of *T.cruzi* which causes *Chagas* disease in humans (Soulsby, 1968; Adam *et al.*, 1971).

2.3.1 *T.cruzi* (*Schizotrypanum cruzi*)

T.cruzi affects man and various species of domestic, wild, and laboratory animals. It is tranmitted by Triatomid bugs.
The disease is common in Central and South America and the southern parts of United States. Man gets infected following contamination of wounds with infected faeces after a bite during feeding by an infected bug (Adam *et al.*, 1971).

The life cycle of *T.cruzi* involves posterior station development. Infected triatomid bugs defaecate frequently while feeding and the infective metacyclic trypanosomes are deposited on the skin. These enter the body through the bite wound as the host scratches. Interestingly, the bugs are more active at night. Apparently, infective metacyclic trypanosomes can penetrate the mucous membranes of the mouth, nose and ear. They penetrate into various tissues, especially the muscle, where they develop and multiply into amastigotes displacing muscle fibres. After reproducing, the amastigotes are transformed into trypomastigotes which invade the peripheral blood. As the disease progresses, the blood forms become scarce or absent. If a suitable vector bites an infected person or animal when the trypanosomes are circulating in the blood, they develop in the midgut of the bug. The trypanosomes become attenuated and assume the epimastigote form. These epimastigotes spread into the hindgut of the insect and metacyclic forms appear in the faeces within at least six days (Soulsby, 1968; Adam *et al.*, 1971).

Morphologically, *T.cruzi* has a small size, curved, with a large kinetoplast that lies close to the posterior end. In fresh blood smears, the trypanosomes are constantly bending to form loops with twisting movement. The trypanosomes are fragile and break when thin blood films are made. Thick blood films are more reliable in diagnosis. Demonstration of the trypanosomes in stained smears under the microscope is successful only during the stage of acute infection.

Serology is the best method used in diagnosis in chronic cases and involves Complement Fixation Test, Haemagglutination Test, and Fluorescent Antibody Test. These tests are found to be reliable (Soulsby, 1968; Adam *et al.*, 1971).

2.3.2 *T.rangeli*

This trypanosome infects hosts similar to that of *T.cruzi*. It is also found in the same geographical areas as *T.cruzi*. It is distinguished from *T.cruzi* due to its long trypomastigote form, and a small kinetoplast that lies some distance away from the pointed posterior end. *T.rangeli* differs from *T.cruzi* in its developmental stages as it develops in the midgut of the bug, passing into the haemocoele and finally penetrates into the salivary glands. The metacyclic trypanosomes are inoculated into the host when the bug feeds. In this regard, it is not typical of stercorarian trypanosomes and is probably non-pathogenic (Soulsby, 1968; Adam *et al.*, 1971). The pathogenicity of salivarian trypanosomes in livestock has been summarized in Appendix I

2.3.3 *T.theileri*

This trypanosome infects cattle. Tabanid flies are suspected to be the main vectors.

The disease caused by *T.theileri* is regarded as non-pathogenic. It is common in cattle and occurs world-widely. Its mode and site of reproduction in the bovine host is uncertain. Tabanid flies are thought to be the biological vectors, but, experimental transmission of the trypanosome by these flies is yet to be proved. The organisms are usually scanty in the peripheral blood and demonstration of the trypanosomes is difficult microscopically. A diagnosis is usually based on inoculating freshly drawn suspected blood into blood agar medium. The epimastigote forms appear in the cultures within 5 to 15 days (Soulsby, 1968; Adam *et al.*, 1971).

2.3.4 *T.lewisi*

This is a trypanosome of rats which is transmitted by fleas. *T.lewisi* is regarded as a non-pathogenic trypanosome that has world wide distribution. It occurs in two phases in rats. During the first few days the developmental stages appear in the blood. They are variable in size and shape and many have epimastigotes. After about a week, trypomastigotes appear and persist in the blood for several weeks (Soulsby 1968; Adam *et al.*, 1971).

2.4 Diagnosis of Trypanosomiasis

2.4.1 Indirect Diagnostic Methods

As a result of the difficulty in detecting trypanosomes in the blood, partly due to antigenic variation, several diagnostic methods have been developed in the diagnosis of the disease.

One of the major problems with serological tests is the lack of specificity of the antigen used. Even when anti-trypanosomal antibodies are detected they do not distinguish between current and previous infections, and cross-reactions occur between some species.

The card agglutination test (CATT) adopted to assist diagnosis of West African human sleeping sickness, relies on the presence of anti-trypanosomal antibody to agglutinate intact, stained preserved trypanosomes. This was found to be useful in the diagnosis of *T.evansi* infections in Water-buffaloes. However, dust particles cause non-specific agglutination, while evaporation in the hot and dry climate renders the method unsuitable for field diagnosis of *T.evansi* infection in camels.

The most successful serological tests for the diagnosis of trypanosomiasis are the indirect fluorescent antibody test (IFAT) and the enzyme-linked immunosobent assay (ELISA). Unfortunately, these antibody assays cannot be used practically to confirm a diagnosis of animal trypanosomiasis. At

best, the result of a single determination is only indicative of exposure to the pathogen. Each of the test systems requires expensive equipment. However, these tests have a role in surveillance of the disease, either before or after a control operation, when screening a population of animals for trypanosomiasis (Connor, 1991).

2.4.2 Recent Developments in Diagnosis

Monoclonal antibodies (MAbs) that distinguish between *T.brucei*, *T.congolense*, and *T.vivax* have opened a new avenue in the diagnosis of African trypanosomiases (Nantulya *et al.*, 1987). When MAbs are used in an antigen-trapping ELISA, diagnosis is sensitive and specific enabling detection of latent infections. However, the test gives some false negative results. This may be due to low levels of circulating antigens at the acute stage of an infection. This necessitates the use of other diagnostic tests in conjunction with the antigen-trapping ELISA. In addition, each serum sample is tested separately with each of the three MAbs. The method is also very sensitive to slight alteration in the quality of reagents and buffers. Interpretation of the result requires a lot of experience and extra care (Connor, 1991).

2.4.3 Field Diagnosis of Trypanosomiasis

This relies on the use of least expensive direct parasitological methods. The methods in use include the use of buffy coat technique especially for surveillance. Thin and thick blood smears are important in routine diagnostic services and are useful in follow up of monitoring treatment and response to the chemotherapeutic agents. However, the interpretation of results is difficult in areas of low tsetse fly density when livestock movement is not controlled. Another disadvantage is the initial cost of purchasing a centrifuge and microscope, as well as recurrent cost of employing a skilled microscopist (Connor, 1991).

2.5 Chemotherapy and control of trypanosomiasis

2.5.1 Chemotherapy of trypanosomiasis:

The high costs of vector control operations and inadequate methods to protect the areas reclaimed from tse-tse fly infestation have led to widespread dependence on trypanocides. The four most important trypanocides in use are diminazene, homidium and isometamidium which are used mainly in cattle, goats and sheep. The fourth compound, quinapyramine was withdrawn from the market in 1977 due to emergence of widespread resistance among trypanosomes in cattle. It was re-introduced in 1985 mainly to treat *T.evansi* infection in camels and horses (Connor, 1991). The drug is also useful in the treatment of African trypanosomiasis in horses and pigs. Despite the fact that suramin (the oldest trypanocide still marketed) is used to treat early cases of human sleeping sickness and *T.evansi* infections in camels, it is ineffective against the other species of trypanosomes. The other trypanocides differ in their activity against the different species of trypanosomes. Diminazene is effective against *T.congolense* and *T.vivax* at the rate of 3.5 mg/kg body weight, but must be used at 7.0 mg/kg body weight or more to treat *T.brucei* infections. The Phenanthridinium related compounds, homidium and isometamidium, are effective against *T.congolense* and *T.vivax* but, less effective against the *brucei* group. There are strain differences and isometamidium is effective against *T.evansi* in some cases (Connor, 1991; Appendix II). Satisfactory treatment of trypanosomiasis requires not only administration of trypanocidal drugs but also adequate nutrition and exercise during convalescence. However, chronic trypanosomiasis often fails to respond to therapy. The ferrokinetic disturbances and accompanying dyshaemopoeisis appear to be irreversible and the affected animals may appear thin and anaemic despite trypanocidal treatment (Connor, 1991). Clinical recovery following treatment results in exposure of trypanosomes to low concentrations of trypanocide, thereby increasing the risk of drug resistance.

2.5.1.1 Attempt to improve efficacy of trypanocides:

The intravenous administration of isometamidium as a 1% w/v solution has been used successfully to treat trypanosomiasis in cattle (Dowler *et al.*, 1989). There is need for care to avoid acute systemic toxic effects and perivascular leakage of the solution. The likely problem associated with this drug and its route of administration is that the period of prophylaxis is likely to be reduced in the absence of a depot of drug at the site of injection. The several local effects of isometamidium injection was reduced by complexing isometamidium with the polyanion dextran sulphate (James, 1978; Aliu and Chineme, 1980). The local tolerance and prophylactic activity of isometamidium-detran complex was found to produce less severe local reactions in cattle and the period of prophylaxis under natural tsetse fly challenge was reduced.

Fluck and Hopkins (1988) made an attempt to prolong the activity and improve the tolerance of diminazene, homidium, and isometamidium by incoporating each of them with liposomal formulations. There was no adverse effect following administration of the phospholipid complex. The reaction to the solution of isometamidium was more marked compared to the less severe reaction at the intramuscular injection site of liposomal isometamidium. Subcutaneous injection of the liposomal isometamidium at the rate of 20 mg/kg body weight produced less severe reaction than that of isometamidium alone at 0.25-0.5 mg/kg body weight. However, duration of prophylaxis was not enhanced with the liposomal preparation of the three compounds (Fluck and Hopkins, 1988). The disadvantages of liposomal isometamidium in cattle include high cost of formulation and instability (Connor, 1991).

2.5.1.2 Toxicity of trypanocides:

Trypanocides have narrow therapeutic indices, hence their use is restricted since their toxicity varies with animal species. Diminazene is toxic to equine, camels and dogs, but may be used safely in cattle, goats and sheep

(Connor, 1991). Apart from systemic toxic effects due to overdose, there may be marked local side effects following drug administration. This is more pronounced when isometamidium is used especially following subcutaneous injection. The trypanocides are in powder form (isometamidium, quinapyramine), granular form (diminazene), or as tableted form (homidium). Therefore, each of the drugs has to be reconstituted in either distilled or boiled cooled water to make up the solution or suspension before use. Severe reaction can occur at the site of injection if normal aseptic precautions are not observed in preparing solutions and giving injections (Connor, 1991).

2.5.2 Control of trypanosomiasis:

2.5.2.1 Chemoprophylaxis against trypanosomiasis:

Several reports have confirmed the efficacy of isometamidium as a prophylactic agent in controling trypanosomiasis in livestock especially cattle in tsetse fly infested areas. The advantage of herd protection with isometamidium over the therapeutic approach was demonstrated (Wilson *et al.*, 1975; Blaser *et al.*, 1979; Logan *et al.*, 1984; Takken *et al.*, 1988). Whitelaw *et al.* (1986) reported that the duration of protection by isometamidium is as long as five months, following their experimental studies. However, numerous factors modify the duration of protection (Ogunyemi and Ilemobade, 1989). It was shown experimentally that the dose of isometamidium has significant effect with higher dose rates conferring longer protection. However, prolonged protection with isometamidium requires extra care since the dosage of the drug cannot be increased greatly because of its toxic effects (Connor, 1991). Normally, the drug is used at 0.5 mg/kg body weight up to 1.0 mg/kg body weight. A dose rate of 2.0 mg/kg body weight was not tolerated by cattle (Connor, 1991).

In practice it is not easy to determine the precise timing of repeat herd treatments. The need to maintain the protective level of isometamidium at all times to prevent re-establishment of trypanosomal infections has to be

weighed against its practicality and economics. Each field situation may require a different approach and the criteria for determining the time of treatment for prophylaxis varies. The decision on when to repeat treatment should not be restricted to parasitological parameters alone but it should incorporate an element of clinical judgement (Connor, 1991). Wilson *et al.* (1975) reported that repeat group treatment with isometamidium may be given when the first animal in a group of twenty regularly monitored cattle becomes parasitaemic. However, the authors concluded that it will be more economical to delay treatment until three out of twenty animals become parasitaemic. In the absence of regular parasitological and entomological monitoring to determine the probable risk or the challenge the animals face in tsetse fly infested areas, repeat treatments should be made at arbitrary three months intervals (Connor, 1991).

Based on the local epidemiology, strategic chemoprophylaxis of trypanosomiasis can be adopted. Cattle entering a tsetse fly infested dry season grazing reserve are to be given prophylactic treatment before exposure to challenge. Also animals in sedentary herds should receive protection before the anticipated onset of high risk (Connor, 1991). The incidence of infection increases immediately after the onset of the rainy season in areas with unimodal rainfall. Njoku *et al.* (1985) and Connor (1989) have demonstrated the efficacy of strategic prophylaxis before the onset of the rainfall (high risk period). Similarly, prophylaxis was provided to village cattle over the high risk period as three treatments with isometamidium at three months intervals, resulting in good control of typanosomiasis and improved productivity (Maloo *et al.*, 1988). Sanative diminazene treatment was included during the period of lowest trypanosomiasis risk.

The beneficial effects of chemoprophylaxis were demonstrated very well by Trail *et al.* (1985) who reported high level of productivity following chemoprophylaxis in Boran cattle, which compared favourably with that of Boran cattle under trypanosomiasis-free ranching conditions in Kenya. Itty

et al. (1988) reported that chemoprophylaxis in East African Zebu cattle under tsetse fly challenge yielded a positive rate of return in productivity. The results of the study indicated improvement on milk production interms of lactation yield and producer prices. Bourn and Scott (1978) reported that cattle for traction were maintained successfully under tsetse fly challenge following chemoprophylaxis. However, resistance to isometamidium as a chemoprophylactic agent emerged and the problem was controlled by sanative use of diminazene at nine months interval. Hendy *et al.* (1988) reported favourable responses of goats to chemoprophylaxis with reduced parturition intervals, higher birth weights, increased kid survival in the perinatal period and increased growth rates of suckling kids of isometamidium-treated dams. In a similar and related study, Connor (1989) showed a proof of a positive economic impact on productivity in goats and sheep following chemoprophylaxis. Kanyari *et al.* (1983) showed the beneficial effect of chemoprophylaxis on fertility in goats through pre-breeding prophylaxis. This improved fecundity, resulting in higher twinning rates. Also, prophylaxis alone given during pregnancy improved birth weights, with bigger and stronger kids (Connor, 1991).

2.5.2.2 Control of trypanosomiasis through vector control

As early as 1949, Morris and Morris introduced a simple type of trap named "*the animal trap*" for controlling tsetse flies in West Africa. They used the trap against *G.palpalis* and *G.tachinoides* and concluded that the traps could not compare in efficiency with selective clearing, although they might be useful as a subsidiary means of tsetse fly control (Morris and Morris, 1949; Ford, 1970). However, only 2 tsetse fly control methods were used with reasonable success before 1950. These included the destruction of larger wild mammals on which tsetse flies were feeding on for their survival, and the modification of the land bush cover by felling trees and clearing the bush so as to destroy the habitat of the tsetse flies. Since 1930, about 10,000 square miles have been free from tsetse fly in Rhodesia by shooting large wild mammals around Zambesi basin (Ford, 1970).

The use of bush clearing, insecticide application, and confinement of shooting wild animal hosts favoured by the *Glossina* has reduced the number of animals killed yearly while at the same time controlling the tsetse fly population since 1955 (Ford, 1970). In recent years, the use of insecticides to control tsetse fly has been on the increase. Pyrethrum sprays in deflying chambers have been in the use and the introduction of powerful residual insecticides such as DDT lead to successful destruction of tsetse flies.

Another control method is that of the sterile male release. Males are rendered sterile by gamma-irradiation and released into a known tsetse infested area. Female tsetse mated by such flies produce non-viable offsprings. This technique has proved effective in controlling tsetse population but is capital intensive and requiring specialized equipment and skilled manpower. This method was anticipated several years ago by Vanderoplank who showed that the closely related tsetse, *G.morsitans* and *G.swynnertoni*, would mate and produce sterile offsprings. However, an attempt to eliminate a small belt of *G.swynnertoni* by releasing large number of *G.morsitans* into it failed, because the introduced species (*G.morsitans*) took up a different niche in the habitat and as such did not mix with the indigenous population. It is partinent to note that chemosterilized males will not do this when introduced into their natural habitat (Ford, 1970). Therefore, irradiation to sterilize male tsetse flies became one of the control measures of *Glossina* species.

2.6 The clinical signs of trypanosomiasis

The clinical signs of trypanosomiasis include fever, anaemia, abortion, weakness, incoordination, hyperesthia, infertility, sterility, coma, loss of weight and lymphadenitis (Fiennes *et al.*, 1946; Fiennes, 1954; Edwards *et al.*, 1956; 1957; Apted, 1970; Omeroid, 1970; Stephen, 1970, Takayanagi *et al.*, 1974; Saror, 1980; Ilemobade and Balogun, 1981; Esievo and Saror, 1983; Njoku, 1985).

2.6.1 Clinico-pathological features of *T.vivax* infection in cattle

During infection with *T.vivax* (strain Y58) four stages were identified by Van Den Ingh *et al.* (1976).

(a) A prepatent period of about one week.

(b) An acute or fulminating disease characterized by an almost continuous, fluctuating parasitaemia, a decrease of the number of thrombocytes, blood serotonin level, packed cell volume (PCV) and Hb, serum albumin and Gamma-globulin fractions, a low serum total liquid content, and an initial rise with subsequent decrease of the blood bradykinin level.

(c) A critical stage, about 4-5 weeks post infection during which the animal may develop a progressive parasitaemia and may die from disseminated intravascular coagulation. At necropsy, microthrombi were a consistent and most significant observation. Other lesions were oedema and haemorrhages in the various tissues and organs, pulmonary oedema and lobular infarction, patches of necrosis in the liver, spleen, adrenals and nephrotic changes in the kidneys. Perivascular mononuclear infiltrations resulting in local granuloma formation in the heart and liver were also observed. Related biochemical changes included increase in blood urea and serum lactate dehydrogenase levels.

(d) A post-critical stage characterized by an ameliorating condition, with few or no trypanosomes in the peripheral blood alternating with relatively high peaks of parasitaemia. This was associated with increased blood bradykinin levels, increasing attempt of PCV and Hb recovery, serum albumin and γ-globulin fractions, number of thrombocytes and blood serotonin level. A return to normal values of

the serum total lipids may occur at this stage. However, moderate to masssive histiocyte, lymphocyte and plasma cells infiltration into the heart (myocardium) led to cardiac insufficiency (Van Den Ingh *et al.*, 1976).

The rise of di-amino laevulinic acid dehydratase levels of erythrocytes during the decline of the PCV and Hb values is an indication for the production of many young erythrocytes and thereby for the haemolytic nature of the naemia (Van Dan Ingh *et al.*, 1976).

Other observed microscopic lesions were centrilobular and paracentral necrosis in the liver, localized erythropoiesis and myelopoiesis, lymphoid hyperplasia in the lymph nodes, mild lymphoid hyperplasia in the spleen and hemosiderosis in the lymph nodes, spleen and liver. Degenerative changes in the heart and skeletal muscle. Proteinaceous fluid (oedema) in the Bowman's space and renal tubules. Erythrophagia in the liver, lymph node and spleen. Intravascular and extravascular trypanosomes were seen (Van Den Ingh *et al.*, 1976).

2.6.2 The reproductive pathology of trypanosomes infected livestock

Various pathological lesions caused by trypanosomes have been reported in the various species of livestock. The lesions have significant effects on the reproductive performance of our livestock and has serious negative effects on our economy, protein requirement interms of meat, milk, growth rate, fertility and fecundity. The results of these are malnutrition and severe economic loses, hence, the need for the review of the effects of trypanosomasis on the reproductive system.

2.6.3 The effects of trypanosomiasis on the estrous cycle and the ovary

Silent estrous, irregular estrous, temporary and complete anaestrus were reported in Yankasa sheep infected with *T.vivax* (Adenowo, 1989). Chronically *T.vivax* infected heifers showed anestrus of up to 6 months (Ogwu *et al.*, 1984). Anaestrous of 100 days was reported in Boran Cows infected with *T.congolense* (Lunckins *et al.* 1986; Llewelyn *et al.*, 1988).

Irregular, short estrous cycles and anaestrous were reported in goats (Luckins *et al.*, 1988). Low fertility was reported in herds of both male and female camels by Guleed and Bornstein (1987). The lesions associated with the reproductive system of the females infected by the various species of trypanosomes were well documented. The overian lesions included cystic degeneration in cattle, goats and sheep infected with *T.vivax* (Vohradsky, 1971; Mutayoba *et al.*, 1985; Isoun and Anosa, 1974a; 1974b). Arrested ovarian function for 6 months post-infection associated with developmental failure of the ovarian follicles and corpora lutea was reported in *T.vivax* infected Zebu heifers (Ogwu *et al.*, 1984). Also, infection with *T.congolense* resulted in stoppage of the cyclical ovarian activities (Luckins *et al.*, 1986; Llewelyn *et al.*, 1988). Atrophic ovaries with multiple follicular cystic degeneration and total absence of corpora lutea were reported in Zebu heifers in a similar experiment (Ogwu and Njoku, 1991). Ovarian dysfunction associated with development of atretic primodial and primary follicles and lack of corpora lutea development were reported in *T.congolense* infected goats (Mutayoba *et al.*, 1988). However, there were reported persistent corpora lutea in goats (Luckins *et al.*, 1986; Llewelyn *et al.*, 1987).

2.6.4 The effects of trypanosomiasis on the vagina, uterus, uterine horns and cervix

Ogwu and Njoku (1991) observed endometritis, glandular atrophy, periglandular cellular infiltration, myometrial atrophy, fibroplasia in the uteria, cervicitis associated with non-secretory desquamated mucosa, vaginitis and necrosis in *T.congolense* infected Zebu heifers. The severity of the lesion was directly related to the duration of the infection. However, in an earlier report, Ogwu *et al.* (1984) observed atony of the uterine horns. Isoun and Anosa (1974a, 1974b) reported infertility and sterility in West African Dwarf sheep and goats infected with *T.vivax*. Deaths of cattle, sheep, goats, and camels during pregnancy or dystocia due to trypanosomiasis was associated with weak or dead offspring or expulsion of congenitally malformed dead foetuses (Ikede and Losos, 1972; Akpavie, 1982; Luckins *et al.*, 1986; Ogwu *et al.*, 1986; Llewelyn *et al.* 1987; Ikede *et al.*, 1988; Anene *et al.*, 1991; Waitumbi and Connor, 1991). Abortions at the various stages of pregnancy, birth of weak offsprings, premature births, birth of anaemic offsprings, low birth weight and neonatal calf mortalities were reported in *T.vivax* and *T.congolense* infected cattle (Boyt, 1971; Ogwu *et al.* 1986; Ogwu and Njoku 1987; Fio-Ngaindiro, 1987; Anene *et al.*, 1991). Abortions, premature births and stillbirths were reported in trypanotolerant breed of cattle infected with *T.congolense* (Djabakou *et al.*, 1985). Reduced kidding percentage was reported in female goats infected with *T.congolense* (Kanyari *et al.*, 1983). Cases of abortions were also reported in buffaloes infected with *T.evansi* (Lor *et al.*, 1986) and camels (Guleed and Bornstein, 1987; Waitumbi and Connor, 1991). Retained placentae following dystocia was also reported in Zebu heifers infected with *T.vivax* (Ogwu *et al.*, 1986).

Interestingly, intra-uterine transmission of *T.vivax* was associated with development of atretic ovaries, reduced numbers of primodial and primary follicles, and lack of corpora lutea development in *T.congolense* infected goats (Mutayoba *et al.* (1988). However, there were reported cases of

persistent corpora lutea in goats (Luckins *et al.*, 1986; Llwelyn *et al.*, 1987). In addition, the trypanosomes were demonstrated in the amniotic fluid in *T.vivax* infected infected sheep and goats (Isoun and Anosa, 1974a; 1974b). In most cases of the transplacental transmission it was associated with placentitis and neonatal death (Sekoni, 1994).

2.6.5 The effects of trypanosomiasis on the testis, epididymis and semineferous tubules

Testicular lesions in *T.vivax* infected bulls including degeneration of the epididymis, semineferous tubules, interstitial Leyding cells, prevention of spermatogenesis associated with lack of epididymal sperm reserve, severe degeneration of the seminal vesicles, bulbo-urethral and prostate glands in *T. congolense* infected bulls have been reported (Sekoni *et al.*, 1990c). *T.congolense* was observed to be more pathogenic than *T.vivax* in experimentally infected Zebu bulls. The severe lesions produced resulted in elevated ejaculation time, poor semen quality (Sekoni *et al.*, 1988; Sekoni *et al.*, 1990b, 1990d). Chemotherapy with homidium chloride at 12 weeks post-infection failed to revert the deteriorated semen characteristics to the pre-infection levels at 18 weeks post-treatment (Sekoni *et al.*, 1991). Grundler and Djabakou (1985), and later Grundler *et al.* (1988) reported the deterioration of semen characteristics following *T.congolense* infection in West African shorthorn bulls with the testicular lesions persisting 47-52 weeks after treatment with diminazene aceturate. An increased scrotal diameter, inflammation, scab formation with alopecia of the scrotum, periochitis, epididymitis, severe testicular degeneration, abnormal spermatogenesis and aspermatogenesis were reported in rabbits, sheep, and goats infected with *T.brucei* (Anosa and Isoun, 1980; Ikede and Akpavie, 1982).

Treatment of the rabbits with diminazene aceturate resulted in incomplete resolution of the genetal lesions. Severe scrotal enlargement, severe degeneration of the testicles, epididymis, and deteriorated sperm

characteristics in bucks and rams infected with *T.vivax* were reported (Isoun and Anosa, 1974a, 1974b; Anosa and Isoun, 1980; Agu *et al.*, 1986; Sekoni, 1992; 1993). Ram semen characteristics have been reported to deteriorate following infection with both *T.vivax* and *T.brucei* (Akpavie *et al.*, 1987). The good quality of ejaculates was obtained 19 weeks post-treatment with diaminazene aceturate. However, *T.brucei* was found to be more pathogenic in rams than *T.vivax* (Ikede, 1979). It was therefore concluded that trypanosomiasis has an important economic impact on the livestock industry in many African countries, manifested through anaemia, cachexia, death, and reproductive disorders involving degeneration of the hypothalamus, pituitary glands and gonads resulting in hormonal imbalance thereby causing disturbances in the normal reproductive processes in both male and female animals. Losses occur through delayed puberty, loss of libido, degenerative changes in the genitalia resulting in production of poor semen or cessation of semen production. In addition, temporary or permanent anaestrus, abnormal estrous cycles, death as a result of dystocia, abnormal pregnancy, premature birth, abortion, low birth weight, stilbirth, intra-uterine foetal infection, neonatal death, congenital malformations in foetuses and other pathogenic effects on foetuses and offsprings. Trypanosome-induced reproductive disorders are of great importance in the tropics especially sub-Saharan African countries where tsetse flies that transmit trypanosomes are endemic. Chemotherapy with trypanocides has little effect in the resolution of genital lesions (Sekoni, 1994).

2.7 The anaemia

Anaemia is one of the most important features of clinical trypanosomiasis in animals and humans. Death due to trypanosomiasis is possibly as a result of severe haemolytic crisis (Fiennes, 1954; Losos and Ikede, 1972; Mackenzie and Cruickshank, 1973; Mamo and Holmes, 1975; Kobayashi *et al.*, 1976). Reports on the mechanisms involved in the development of the

anaemia of trypanosomiasis are numerous and variables. These mechanisms include:-

2.7.1 Haemodilution as a cause of the anaemia in trypanosomiasis

Haemodilution was reported to be one of the contributing factors to the anaemia of animal trypanosomiasis. These observations included increase in blood volume (Anosa and Isoun, 1976), increase in volume of plasma (Fiennes, 1954; Naylor, 1971; Maxie and Valli, 1978), absence of change in total blood volume (Clarkson, 1968), and absolute fall in circulating erythrocytes (Mamo and Holmes, 1975; Anosa and Isoun, 1976). However, these observations were challenged by other researchers (Jennings *et al.*, 1975; Ingh *et al.*, 1976; Dargie *et al.*, 1979a, 1979b). The phenomenon of haemodilution was regarded not to be a feature of animal trypanosomiasis by Dargie *et al.* (1979a, 1979b); 56_{Fe} used to measure plasma volume was found not to equilibrate with the plasma space and over-estimated the plasma volume of anaemic animals (Dargie, 1978; Dargie *et al.*, 1979a, 1979b).

2.7.2 Response by the haemopoietic system as a contributing factor to the cause of the anaemia in trypanosmiasis

Bone marrow responses in trypanosomiasis has been studied. Depression of erythropoiesis with defects in the bone marrow growth has been observed at early stages of *T.brucei* infection in rabbit (Boycott and Prince-Jones, 1913), likewise degenerative changes in the bone marrow of *T.congolense* infected cattle. Thus indicating dyshaemopoiesis (Fiennes, 1954; Naylor 1971; Losos and Ikede, 1972).

Active erythropoiesis in rabbits infected with *T.brucei* (Boreham, 1968) and *T.congolense* infections (Holmes and Jennings, 1976), rats infected with *T.brucei* (Jennings *et al.*, 1974), and *T.evansi* (Assoku, 1975). Active erythropoiesis was also reported in *T.congolense* infected sheep (Mackenzie and Cruickshank, 1973), *T.vivax* infected goats (Saror, 1980), and cattle

(Van Den Ingh *et al.*, 1976). Erythroid responses were more marked in cattle infected with *T.congolense* than those infected with *T.vivax* (Maxie *et al.*, 1976; 1979). These latter reports cast doubt on the role of dyshaemopoiesis in the anaemia of animal trypanosomiasis.

2.7.3 The role of haemolytic factors as the cause of the anaemia in trypanosomiasis

Trypanosomes may exert a direct haemolytic action on the erythrocytes by generating potentially heomolytic factors on autolysis (Landsteiner and Raubitscheck, 1907; Nguyen Huan, 1975). Landsteiner and Raubitscheck (1907) showed that *T.equiperdum* could release a haemolysin after overnight incubation in the cold. The investigators showed that the released haemolysin was a lipid and suggested that it might be fatty acid in nature.

Huan *et al.* (1975) demonstrated that autolysed *T.brucei* was also haemolytic. However, it was indicated that the haemolytic factor in this case was probably a small protein. A toxic pronase-sensitive protein of molecular weight 10,000 daltons, a haemolytic factor from the serum of *T.brucei* infected mice and supernatant of the trypanosome suspensions has been extracted.

Tizard *et al.* (1977) showed that the haemolytic material from *T.congolense* consisted of a mixture of free fatty acids (stearic, palmitic, oleic and linoleic) and possibly some lysolecithin generated by the action of trypanosome phospholipase A on endogenous phosphatidyl choline. However, *T.lewisi*, being devoid of phospholipase A, does not generate free fatty acids and is therefore non-haemolytic (Tizard *et al.*, 1977). *T.congolense* releases haemolytic free fatty acids, on autolysis, of which the most potent is linoleic acid. These fatty acids can lyse washed rat and bovine erythrocytes *in-vitro*. Autolysed *T.congolense* will cause increased fragility of erythrocytes in whole rat blood but not in whole bovine blood. The lack of effect of these fatty acids on bovine erythrocytes tends to be confirmed by the failure of this materials to influence the survival of

these cells *in-vivo*. Calculations based on the amount of the free fatty acids required to cause haemolysis *in-vivo*, also confirmed that this mechanism of erythrocyte destruction is probably not important in bovine trypanosomiasis since both the fatty acids and lysolecithin are rapidly esterified and bound to albumin *in-vivo* (Tizard *et al.*, 1978). This mechanism may be effective in the lysis of erythrocytes in cappillaries were large number of *T.congolense* may accumulate and their lysis may cause large release of fatty acids locally to haemolyse erythrocytes in the capillaries.

It is interesting to note that as early as 1954, Fiennes observed that erythrocytes from normal bovine blood were haemolysed when incubated with plasma from trypanosome infected animals. The investigator suggested possible involvement of haemolytic factors in the anaemia associated with bovine trypanosomiasis. This was supported by the appearance of erythrocytes in blood smears made at the time of crisis, by positive indirect Van den Bergh reaction, presence of urobilinogen in the urine of extreme cases, improved reticulocyte counts following treatment. The haemolytic factor was present intermittently, and is always present terminally with highest concentration at the time of trypanolysis. Fiennes (1954) reported that while haemolysis might be associated with the acute course of the disease, or in the fatal termination of the disease, the haemolytic factor was not regarded as the underlying cause of the progressive anaemia. This factor was found to be produced by other trypanosomes, eg *T.vivax* infection in cattle, but, its role in the development of the anemia was not ascertained (Murray *et al.*, 1977).

2.7.4 Immunological basis as a cause of the anaemia in trypanosomiasis

It was suggested that the increased rate of destruction of erythrocytes may be mediated by immunological mechanisms (Herbert and Inglis, 1973; Kantzier *et al.* 1974; Kobayashi *et al.*, 1976). Repeated intravenous injections into rats with over 100 ug/100g body weight of soluble *T.evansi* antigen resulted

in moderately severe, probably antibody mediated haemolytic anaemia. It was then hypothesized that development of the anaemia associated with *T.evansi* infection might be immunologically mediated (Assouku, 1975). This was supported by the presence of trypanosme antigen-antibody-complement complexes on the surfaces of erythrocytes of calves infected with *T.congolense*, leading to the speculation that the immune complexes caused the elimination of affected erythrocytes resulting in clinical anaemia (Kobayashi *et al.*, 1976). Immunoglobulins bound to erythrocytes of 13 infected calves were detected by a direct antiglobulin test 7 to 10 days post-infection. The reation was strongest between 3 to 9 weeks post-infection. Immunologbulins were eluted from erythrocytes reacting positively to the test. Of 74 elutes, 16 contained IgM and IgG. Antibody activity of the eluates against *T.congolense* was demonstrated by complement fixation, indirect haemagglutination and indirect antiglobulin tests. It was then postulated that the anaemia in mammalian trypanosomiasis and especially in *T.congolense* infected calves was of immunological origin (Kobayashi *et al.*, 1976). IgG and IgM classes were separated from the sera of mice infected with *T.congolense*, and both were associated with agglutination reaction, formation of variant serotypes, and protection against infection (Hudson *et al.*, 1976; Takayanagi and Enriquez, 1973; Mansfield, 1978). Other circumstantial evidences for the immunological basis of the anemia in animal trypanosomiasis include elevation of IgM (Clarkson, 1968; 1976 Clarkson *et al.*, 1975; Kobayashi and Tizard, 1976; Moulton and Sollod, 1976; Tabel *et al.*, 1978) and complement fragments on cell surfaces (Kobayashi and Tizard, 1976; Nielsen *et al.*, 1978a, 1978b, 1978c) during the disease. Antibodies to certain complement components, eg C3, which are mainly of the IgM class (Eisen, 1974) were elevated following infection with pathogenic African trypanosomes (Ingram and Soltys, 1960). Kobayashi *et al.* (1974) showed that complement was not involved in the attachment and ingestion of *T.gambiense* by rat macrophages and suggested that the role of complement in the pathogenesis of the anaemia might be restricted to the haemolysis.

It was shown that erythrocytes of rabbits infected with *T.brucie* and of calves infected with *T.congolense* were antiglobulin positive. Occassionally, some of the animals possess erythrocytes with detectable surfae C3. Immunoglobulins were eluted from these erythrocytes and were shown to possess antibody activity directed against trypanosomes (Herbert and Inglis, 1973; Kobayashi *et al.*, 1976; Nielsen *et al.*, 1978d). It was shown that host erythrocytes adsorb trypanosome antigen *in-vivo* (Herbert and Inglis, 1973) and as a result of the immune response to the trypanosomes, the coated erythrocytes were subsequently eliminated (Herbert and Inglis, 1973).

It may be concluded that immunoglobulins and complement opsonize desialylated erythrocytes (Jancik *et al.*, 1978; Kuster and Schauer, 1981; Muller and Schauer, 1983) which are produced in acute bovine trypanosomiasis (Esievo, 1979; Esievo *et al.*, 1982) leading to erythrophagocytosis. However, the mechanisms of destruction of erythrocytes are complex. It varies with the species of the tyrpanosome, the host, and the stage of infection. A more severe anaemia, for instance, occurs in mice with *T.congolense* than *T.brucie* infections (Ikede *et al.*, 1977).

Jennings *et al.* (1974) suggested that the rapid onset of anaemia in trypanosomiasis might be due to damage to erythrocytes as a direct or indirect result of trypanosome infection. The investigators were of the opinion that immunological mechanisms might have been significant only after 7 days of infection, when an immune response might have been mounted by the host. This is sequele to their observations on the anaemia developed in *T.brucei* infected mice within 4 days post-infection. The pathogenesis of the anaemia earlier than 7 days post-infection with pathogenic trypanosomes was therefore, yet to be established.

2.7.5 Role of immunosuppression in the aggrevation of the termination of trypanosomiasis

It is pertinent to highlight the role of immunosuppression as a possible factor contributing to the pathogenesis of trypanosomiasis, although, the present study is mainly investigating the role of erythrophagocytosis and lactose infusion in trypanosomiasis. Immunosuppression simply means prevention of the occurrence of immune reaction (Roper, 1978). Goodwin *et al.* (1972) reported the phenomenon of immunosuppression in mice and rabbits infected with *T.brucei*. They observed the depression of antibody response of the host to unrelated antigens. For over 3-4 weeks of the infection in mice, the immunosuppression became progressively greater and accompanied the increasing parasitaemia. Greenwood *et al.* (1971) suggested that immunosuppression was brought about by a failure of the antigen processing mechanism of the macrophages. Most attention has hitherto been directed to alterations in the humoral response. However, Allt *et al.* (1971) noted that, concurrent trypanosome infections reduced the severity of induced experimental allergic neuritis in rabbits, suggesting that cell-mediated responses were also affected. Goodwin *et al.* (1972) reported that the indications of antiglobulin reactivity obseved in the experiment, may reflect the extensive tissue damage which occurs during infection resulting in the alteration of modified host tissue proteins which act as antigens. The authors reported that patients with chronic trypanosomiasis show little resistance to concurrent bacterial pneumonias and septicaemias. It has been recognized since the beginning of the century that patients with sleeping sickness have an increased susceptibility to bacterial infections, especially pneumonia the common cause of death. Secondary infections occur in trypanosome infected cattle and may hasten their death (Greenwood, 1976). An impaired antibody response to *Salmonella typhi* vaccine was found in a study of immune competence in 38 patients with *T.gambiense* infection. The patients failed to respond to normal skin testing with purified protein derivative and *Candida* antigen. Fewer trypanosomiasis patients than controls responded to skin sensitization with dinitrochlorobenzene

(Reed *et al.*, 1972). Greenwood (1974a) reported that, in man, as in experimental animals, malaria and trypanosome infections lead to an impaired humoral immune response to some but not all antigens. In addition, impairment of cell-mediated immunity has been found in patients with sleeping sickness. The suppression of humoral immunity found in patients with sleeping sickness was not as marked as that observed in experimental animals infected with *T.brucei*, perhaps because *T.brucei* infection leads to a much heavier parasitaemia than *T.gambiense* in man (Greenwood, 1974a).

The pathogenesis of the immune defects has not been clearly delineated. Defective function of macrophages, B-lymphocytes, T-lymphocytes and breakdown in the normal control mechanisms of the immune response were postulated (Greenwood, 1976, Nantulya *et al.*, 1982). However, antigen processing by the macrophages is unlikely to be an important factor as transfer experiments have shown that peritoneal macrophages from *T.brucei* infected mice can process antigen normally (Murray *et al.*, 1974a). Defective function of B-lymphocytes has been implicated as the cause of the humoral defect in trypanosomiasis but, this is unlikely to be a major factor as lymphocytes from an infected animal can successfully reconstitute humoral immunity in a lethally irradiated recipient (Murray *et al.*, 1974a; Jayawardena and Waksman, 1978). Histological studies have shown profound disturbances in the anatomy of the spleen and lymph node of trypanosome infected animals (Urquhart *et al.*, 1972). In the early stages of infection, proliferation of plasma cells and large mononuclear cells was observed with replacement of normal lymphoid tissue. These changes were associated with an increased uptake of I^{125} iododeoxyuridine. Later, lymphoid atrophy and fibrosis may occur. Depletion of T-lymphocytes as a result of these changes could directly impair cellular immunity and because of the helper function of T-lymphocytes in the antibody response to some antigens, could also affect humoral immunity. However, as noted above, infected mice have shown a diminished antibody response to the T-cell dependent antigen lipopolysaccharide. Defective control of the immune

response was also implicated (Murray *et al.*, 1974; Assoku *et al.* 1977; Sacks and Askonas, 1980; Sacks *et al.*, 1980; Askonas *et al.*, 1979; 1980; Musoke *et al.*, 1981; Araujo-Jorge and Souza, 1984; Askonas, 1985).

In this case, immunoglobulin production by cells of the B-lymphocyte series can be influenced by both helper and suppressor T-cells. It has been suggested by Terry *et al.* (1973) that defect in this control mechanism, perhaps brought about by a selective depletion of T-cells as described above, could be the main immunological defect in trypanosomiasis. This attractive hypothesis also provides a rotational explanation for the excessive IgM production which is such a characteristic feature of African trypanosome infections. it is unlikely that any single factor is responsible for the immune defect of trypanosomiasis. it is much more likely that several of the mechanisms discussed above are involved. It has been clearly shown that, what ever the mechanisms involved, the immune defect depends upon the presence of viable trypanosomes. Immune competence is rapidly restored on treatment and occurs long before correction of the pathological changes of the infection could have occurred. This observation suggests the production by live trypanosomes of some inhibitory factors, but it has so far not been possible to demonstrate this factor in the sera of infected animal (Mansfield and Wallace, 1974; Ortiz-Oritz, 1976; Ramos *et al.*, 1978). Suppression of both cellular and humoral immunity is a frequent accompaniment of African trypanosome infections. It is unlikely that any single factor is responsible for the immune defects of trypanosomiasis. It is much more likely that several of the mechanisms discussed above are involved. It has been clearly shown that, whatever the mechanisms involved, the immune defects depend upon the presence of viable trypanosomes. Immune competence is rapidly restored on treatment and occurs long before correction of the pathological changes due to the infection. These observations suggest the production by live trypanosomes of some inhibitory factors, but it has so far not been possible to demonstrate it in the sera of infected animals (Mansfield and Wallace, 1974; Ortiz-Ortiz, 1976; Ramoz *et al.*, 1978). Suppression of both cellular

and humoral immunity is a frequent accompaniment of African trypanosome infections. The exact nature of the immune defect was yet to be known (Greenwood, 1976).

2.8.1 The life-span of the erythrocytes and its sialic acid content

It was reported as early as 1940 that young reticulocytes migrate more slowly under the influence of an electric field than do erythrocytes (Yaari, 1969). It was reported later by Rottino and Angers (1961) that the mobility of the red blood cells obtained from the umbilical cord was higher than that of infants 48 hours after birth. It has been reported by Danon and Marikowsky (1966) that the difference in the mobility of the erythrocytes was as a result of difference in the electric mobility of extremely young and old age group of cells from the same blood samples. Yaari (1969) reported that young red blood cells have a higher negative charge than the old red blood cells and that as red blood cells age their negative charge is progressively reduced, supporting the above observations. Also Marikowsky *et al.* (1966) reported that young red blood cells migrate in an electric field more rapidly than the old red blood cells of the same blood sample. Yaari (1969) reported that the older the red blood cells the slower they migrate in the electric field.

Durocher *et al.* (1975) reported that the role of erythrocyte membrane sialic acid in the erythrocyte survival was not clear, although there was evidence for a reduction in the sialic acid and surface charge in older erythrocytes. The surface charge of human, rat and rabbit erythrocytes was reduced by removing sialic acid with neuraminidase (sialidase). The reduction in the sialic acid content correlated with decreases in electrophoretic mobility associated with the loss of periodic acid shift (PAS) staining of the erythrocyte membrane glycoproteins on the polyacrylamide gels. There was exposure of the galactose moiety. It was also reported by Durocher *et al.* (1975) that Cr^{51} labelled erythrocytes survival in rats and rabbits showed rapid clearance of desialylated

erythrocytes with sequestration by the liver. This suggested that reduction in erythrocyte sialic acid is a mechanism of erythrocyte destruction and may be important in erythrocyte senescence (Durocher *et al.*, 1975). Most of the erythrocytes were cleared by the liver. The defect induced by desialylation is probably not subtle (Durocher *et al.*, 1975).

The significant increase in the rate of erythrocytes destruction with a consequent shortening of the erythrocyte life-span was well documented. Mamo and Holmes (1975) reported that the half-life of 51Cr-labelled erythrocytes was 216 ±47 days in control Zebu calves while in *T.congolense* infected calves it was 142 ±33 days. The significant shortening of erythrocyte life-span due to the accelerated loss of erythrocytes from circulation could account for the anaemia observed in *T.congolense* infected calves. Mamo *et al.* (1977) reported that the mean time for Cr^{51}-labelled red cell activity to fall by 50% was 3.7 ±0.7 days in *T.congolense* infected calves compared to 6.1 ±0.8 days in the control calves. It was demonstrated that the half-life of Cr^{51}-labelled red blood cells in calves infected with *T.congolense* dropped from an initial normal value of 10 days to 4.7 days at about 30 days after infection, followed by gradual return towards normal at about 12 weeks. Thus, red-cell life-span was roughly halved during the acute phase of the infection (Valli *et al.*, 1978). The erythrocyte destruction occurs largely in the liver, in which extensive erythrophagocytosis had been observed (Mackenzie and Cruickshank, 1973; Mamo and Holmes, 1975; Valli and Forshberg, 1979).

2.8.2 The sialic acid content of erythrocytes in trypanosomiasis

Sialic acid, the acylated neuraminic acid occurs in reasonable quantities on the surface of the erythrocytes in various species of animals (Eylar *et al.*, 1962; Seaman and Uhlenbruck, 1963). *Vibrio cholera* neuraminidase-desialylated Cr^{51}-labelled erythrocytes was cleared rapidly from the circulation of rats and rabbits followed by subsequent sequestration in the liver. This, suggested that reduction in the erythrocyte surface sialic

acid was one of the mechanisms of senescence and destruction (Durocher *et al.*, 1975). An elevated level of serum sialic acid was reported following infection of cattle with *T.brucei* and *T.vivax* (Esievo *et al.*, 1982). Banks (1979; 1980) reported that *T.congolense* bound to bovine erythrocytes *in-vitro*, via neuraminic acid receptors on the erythrocytes, leading to damage to the erythrocytes. Esievo (1979; 1983) reported that *T.vivax* has hydrolysed sialic acid *in-vitro* and the activity of the neuraminidase is proportional to the concentration of the trypanosomes. It was reported by Esievo *et al.* (1982) that the decline in the erythrocyte surface sialic acid and increase of free serum sialic acid concentrations in *T.vivax* infected cattle corresponded to the time of appearance of circulating trypanosomes with lowest values red cell sialic acids at the initial peak of parasitaemia. Recovery from decreased level of the sialic acid content was observed at the end of the parasitaemic phase and the PCV remained stable then. The authors indicate that, it appears that the loss of the erythrocyte surface sialic acid in trypanosome infected cattle was related to the intensity of the circulating parasites (Esievo *et al.*, 1982). It was demonstrated that trypanosome produce neuraminidase *in-vitro* and the enzyme was shown to have cleaved off the erythrocyte surface sialic acid in different species (Eylar *et al.* 1962; Seaman and Uhlenburck, 1963; Durocher *et al.*, 1975; Esievo, 1979). Esievo *et al.* (1982) postulated that the early anaemia observed in infected animals may be attributable to the activities of the circulating trypanosomes which produce neuraminidase. The neuraminidase cleaves off surface sialic acids from erythrocytes thus rendering them more prone to erythrophagocytosis. The slight increase in free serum sialic acid concentrations in both the infected and control calves at the period of greatest erythrocyte surface sialic acid loss and peak parasitaemia may be noteworthy despite the fact that there was no significant difference between the infected and control calves (Esievo *et al.*, 1982).

2.8.3 The role of sialic acid in trypanotolerance in animal trypanosomiasis

It has been known for many years that N'dama and Muturu cattle, which are dwarf breeds in West Africa are less susceptible to infection with trypanosomes than West African Zebu cattle (Mason, 1951). The nature of the resistance i.e. trypanotolerance which is the natural resistance to trypanosomiasis is not well understood (Mason, 1951). Choquel (1969) revieved the experience with N'dama cattle in several African countries and considered that their tolerance was natural rather than acquired. Weitz (1970) suggested that the resistance of the dwarf breeds probably results from a more efficient immune response to trypanosome infection compared to Zebu.

The degree of resistance that the animals develop seems to depend on factors such as initial exposure of the dam and the new-born calf to a low grade trypanosome challenge, continual exposure to the same antigenic types of trypanosomes and maintenance of reasonable conditions of husbandry (Chandler, 1952; Desowitz, 1959).

In recent years, it was reported that the relative trypanotolerance of the N'dama breed of cattle may be attributable to the possession of a 7-fold increase in erythrocyte surface sialic acids compared to the Zebu breed (Esievo *et al.*, 1986). Esievo *et al.* (1990) showed that erythrocytes of both the trypano-susceptible zebu (White Fulani) and the trypanotolerant N'dama breeds of cattle have sialic acids which coincided with N-acetylneuraminic acid during polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). The N'dama erythrocytes had another type of sialic acids (with estimated molecular weight of 30,000) trailing behind the N-acetylneuraminic acid. However, the additional band was absent in the Zebu breed (Esievo *et al.*, 1990). the existence of the additional type of sialic acid (Esievo *et al.*, 1990) might account for the higher N'dama erythrocyte sialic acid concentrations (Esievo *et al.*, 1986) and the relative trypanotolerance of these breed of cattle. In our recent studies, it was found that the erythrocyte surface

sialic acid concentrations were 25.4 ±5.5 mg/dl and 5.9 ±0.97 mg/dl in N'dama and Zebu animals, respectively. In N'dama, O-acetyl and glycolyl groups were present in concentrations of 16.4 ±4.3 mg per dl and 12.8 ± 2.9mg per dl, respectively, whereas the corresponding values in Zebus were 2.8 ± 5.0mg per dl and 7.6 ± 1.7mg per dl, respectively. The differences between N'dama and Zebu cattle in surface sialic acids and in O-acetyl and glycolyl groups were significant. N'dama erythrocyte sialic acids had more O-acetyl than glycolyl groups while those of Zebus had less O-acetyl than glycolyl groups. These findings may be relevant to the trypanotolerance of N'dama cattle (Shugaba *et al.*, 1994), since it was observed that the protective nature and the rate of hydrolysis of sialic acids by sialidases depend on the type and concentration of side groups viz O-acetyl; glycolyl (Reuter *et al.*, 1983; Schauer *et al.*, 1984).

O-acetyle groups in sialic acid hinder rapid hydrolysis of the sialic acid by sialidases and the protective nature of this variety was attributed to the occurrence of 9-O-acyl-N-acylneuraminic acid in erythrocyte membranes (Reuter *et al.*, 1983; Schauer *et al.*, 1984). The glycosidic linkage of 4-O-acetyle-N-acylneuraminic acid is resistant to the action of sialidases (Schauer *et al.*, 1974; Schauer, 1978; Veh *et al.*, 1979). Sialic acid becomes susceptible to the action of sialidases only after the release of the 4-O-acetyl group, this indicates that the resistance is due to the presence of the substituent in the fourth position of the neuraminic acid molecule (Schauer *et al.*, 1974). Sialic acids with O-acetyle or ester groups at C-7- or C-9 of the neuraminic acid molecule are released by sialidases at reduced rates (Busther *et al.* 1974; Schauer, 1978; Reuter *et al.*, 1983).

The observation made of a much higher concentration of O-acetyle groups in N'dama than in Zebu erythrocyte sialic acid type may account for the higher concentration of the total erythrocyte surface sialic acid in N'dama than in Zebu cattle (Shugaba *et al.*, 1994). The higher concentration of the O-acetyle group in N'dama sialic acid as observed by Shugaba *et al.* (1994) supports an earlier report of the existence of another band or type of sialic acid on the erythrocytes of N'dama (Esievo

et al., 1990). Based on the previous reports that O-acetylated sialic acids are generally resistant to rapid hydrolysis by sialidases (Schauer, 1978; Schauer *et al.*, 1983, Veh *et al.*, 1979), that sialidase activity is related to the number of parasites (Esievo, 1983) and the severity of the anaemia in bovine trypanosomiasis is related to the intensity and duration of parasitaemia (Dergie *et al.*, 1979b) the finding of a much higher O-acetyl group concentration in the erythrocyte sialic acid of the trypanotolerant N'dama cattle (Shugaba *et al.*, 1994) supports the observation that N'dama differ from Zebus in requiring a much higher trypanosome parasitaemia to produce same degree of anaemia (Murray *et al.*,1977).

Based on the above observations it is possible that the N'dama achieve their trypanotolerance partly through a reduced rate of the release of erythrocyte surface sialic acids by the trypanosome sialidase, due to the occurrence of higher concentrations of O-acetyl groups in their sialic acids and due to additional type of sialic acid on the N'dama erythrocytes (Esieve *et al.*, 1990; Shugaba *et al.*, 1994).

2.8.4 The possible role of sialic acids in trypanosomes antigenic variation:-

Antigenic variation is important in the survival of trypanosomes in their hosts. This is achieved by the trypanosomes through development of different antigens on their surface thereby evading the immune defense mechanism of the host. Antigenic variation also has genetic basis (De Gee *et al.*, 1979; Reinwald *et al.*, 1979; Liu *et al.*, 1979; Reinwald *et al.* 1983). This review is not intended to cover the vast majority of findings on the biological, biochemical and genetical basis of the antigenic variation. It is intended to provide information on the relationship between the sialic acids and antigenic variation of tryponosomes.

The evasion mechanism of the immune system by trypanosomes is achieved by changing the glycoproteins pattern of the cell surface which may be related to sialic acid activity. This is due to the fact that sialic acids have been implicated as potential determinants of

differentiation antigens (Schauer, 1983). In both animal and human trypanosomiasis, research findings that may have obvious relevance to the sialic acids and trypanosome antigenic variation are available. *Trypanosome congolense* was shown to be bound to bovine erythrocytes through the sialic acids receptors on the erythrocytes (Banks, 1979). *Trypanosoma congolense* has sialic acids which are responsible for the charge heterogeneity of the variant surface glycoproteins (Reutenberg *et al.*, 1981). *Trypanosoma cruzi*, the etiologic agent of Chagas, disease in south America has its main type of host-immune escape mechanism, it has sialic acids even though it doesn't appear to be capable of synthesizing sialic acids from their precursors (Shauer *et al.*, 1983). Also the production of sialic acid-cleaving enzyme (sialidase) by *T. vivax* (Esievo, 1979) and *T. cruzi* (Pereira, 1983) have suggested that the enzyme is of great importance in the pathogenesis and pathophysiology of bovine trypanosomiasis (Esievo *et al.*, 1982) and Chagas' disease of human (Pereira, 1983; Pereira and Hoff, 1986).

2.8.5 The role of erythrophagocytosis as a cause of the anaemia in animal trypanosomiasis in relation to surface sialic acids removal:-

A review of the role of erythrophagocytosis in the anaemia due to trypanosomiasis was delayed till now probably, because of its relationship to removal of erythrocytes surface sialic acids. The phenomenon of erythrophagocytosis as contributing to the anaemia in animal trypanosomiasis was reported by (Mackenzie and Cruickshank, 1973; Mackenzie *et al.*, 1978; Fifrer and Askonas, 1982; Anosa and Kaneko, 1983ab). The authors reported that both the haemolysis of erythrocytes and erythrophagocytosis alternately act to maintain the animal in a state of anaemia and to foil compensatory attempts by the bone marrow. Unless the immune response of the host is able to keep the parasitaemia at low level, it would appear that there is little chance of the anaemia being successfully alleviated. Haemolysis is dependent on high level of parasitaemia and subsequent erythrophagocytosis depends on the presence of

sufficient antigen (Mackenzie *et al.*, 1978). Mackenzie and Cruickshank (1973) observed that, the phenomenon of erythrophagocytosis was noted only at the acute phase of trypanosomiasis. It was associated with rapidly declining total erythrocyte count, haemoglobin and packed cell volume (haematological crisis) and erythrophagocytosis was not detected in chronic cases. According to Mackenzie and Cruickshank (1973), the most likely cause of erthrophagocytosis were:

i) The coating of erythrocytes with antigenic material derived from the trypanosomes, thus converting the erythrocytes to foreign protein.

ii) Production of defective erythrocytes, through increased osmotic fragility of the erythrocytes.

iii) An auto-immune reaction. Leukophagocytosis was also reported in the liver of animals infected with trypanosomes as early as 1907 by Laveran and Mesnil (1907).

Recent investigation by Nohle *et al.* (1982) revealed that carbohydrate may be covalently, bound to protein to form glycoproteins. The carbohydrates may combine with lipids to form glycolipids, the glycolipids and glycoproteins with their component oligosaccharides are referred to as glycoconjugates. The glycoconjugates have structural functions and some of them coat cell surfaces. Sialic acids occur as terminal sugars in a variety of the glycoconjugates and oligosaccharides. The sialic acids have been found to occur in serum and on the surface of the erythrocytes of mammals (Eylar *et al.*, 1962; Muller *et al.*, 1981; Esievo *et al.* 1982). Binding of desialylated erythrocytes via exposed *B*-specific residues to *B*-specific lectin on the cell membrane of macrophage is necessary for subsequent erythrophagocytosis (Muller *et al.*, 1981; Muller and Schauer, 1983).

It was reported by Muller *et al.* (1981) that bacterial sialidase treatment exposes B-galactose residues of the membranes of erythrocytes. Interaction of the cells with a *B*-galactose-specific lectin on the liver Kupffer cells

and spleen macrophages resulted in binding, rapid sequestration of the sialidase treated erythrocytes from the blood stream and erythrophagocytosis. Goldman and Cooper (1975) reported that lectin stimulated both binding and erythrophagocytosis. Binding via demasked erythrocyte surface *B*-galactosyl residues to the lectin on the macrophage cell membrane is necessary for erythrophagocytosis (Muller *et al.*, 1981; Muller and Schauer, 1983). Kolb and Kolb-Bachofen (1978) inhibited binding between desialylated erythrocytes and macrophages by preincubation of the macrophages with D-galactose and related sugars. The binding was also inhibited by *B*-D-galactosyle containing glycoproteins including lactose (Kolb and Kolb-Bachofen, 1978). Lactose is a disaccharide, it has galactose and glucose. The galactose part competes with the exposed *B*-galactose on the cell membrane of the desialylated erythrocytes for the lectin on the Kupffer cells and binds to it competitively leaving the denatured erythrocytes floating. Binding of desialylated erythrocytes to macrophages did not depend on the metabolic stage of the macrophages (the Kupffer cells), since clusters (rossets) were formed at 0^{oc} and 37^{oc} by both living and dead cells (Kolb and Kolb-Bachofen, 1978).

Erythrophagocytosis is playing a very important role in the maintenance of anaemia in trypanosomiasis. However, not much work has ben done to study erythrophagocytosis in an experimental infection. *Trypanosoma vivax* produces neuraminidase which cleaves off silic acids from the surface of erythrocytes (Esievo, 1979; Esievo *et al.*, 1982) to expose galactose (Muller *et al.*, 1981; Muller and Schauer, 1983) thereby denaturing the erythrocytes for sequestration and destruction. In view of the above, this project was designed to investigate the role of Kupffer calls surface lectin in erythrophagocytosis during acute bovine trypanosomiasis (*T. vivax* infection).

CHAPTER THREE

3.0 THE ROLE OF KUPFFER CELL SURFACE LECTIN IN ERYTHROPHAGOCYTOSIS FOLLOWING ACUTE RUMINANT *Trypanosoma vivax* INFECTION.

3.10 Introduction

Reduction of erythrocytes life-span and erythrophagocytosis is consistent contributory factors in the aetiology of the anaemia of cattle trypanosomiasis (Mackenzie and Cruickshank, 1973; Valli *et al.* 1978; Valli and Forsberg, 1979. Dargie *et al.*, 1979ab). Significant cleavage of erythrocytes surface sialic acids during peak trypanosome parasitaemias and thence highest production of trypanosome sialidase (Esievo; 1979) were incriminated in the shortening of erythrocytes life-span and erythrophagocytosis of bovine trypanosomomiasis (Esievo *et al.*, 1982). Much support for this was found in the numerous quantitative and qualitative biochemical differences between the erythrocytes sialic acids of the trypanosusceptible Zebu cattle and the trypanotolerant Ndama cattle, which also produced favourable explanation for latter's trypanotolerance(Esievo *et al.*, 1986; Esievo *et al.*, 1990; Shugaba *et al.*, 1994).

Earlier, it was postulated that at peak parasitaemia, trypanosome sialidase cleaves off erythrocytes surface sialic acids significantly causing physico-chemical damages on the desialylated erythrocytes rendering them more prone to premature phagocytosis and destruction by the reticulo-endothelial system (Esievo *et al.*, 1982). We have very recently shown that these physico-chemical changes are in fact galactose residues exposed on the membranes of erythrocytes in the *T. vivax* infected cattle, confirmed by the binding and rosetting of such desialylated erythrocytes, by using freshly isolated rat peritoneal macrophages (Omage, 1996, personal

communications) Similar erythrocytes galactose exposure was achieved with bacterial commercially-produced sialidase (Jancik *et. al* 1978; Kolb and Kolb-Bachofan, 1978; Muller *et al.*, 1981; Muller and Schauer, 1983).

It appears that homologous macrophages, particularly liver Kupffer cells in trypaosome infected animals may operate an accelarated rosetting of desialyalted erythrocytes for onward erythrophagocytosis in *T. vivax* infected rumiants. This partly involves the liver Kupffer cells surface lectin, binding onto the galactose exposed on red cell membranes afer desialylation.

3.2.0 MATERIALS AND METHODS

3.2.1 Experimental Animals:

Four (4) young adult goats of about 12 months old and 4 cattle averaging about 18 months old were used for this project. These animals were part of another experiment where they were experimentally infected with *Trypanosoma vivax* by jugular venipuncture. All the experimental animals were screened for any detectable systemic infections, dewormed with albendazole and tick bathed with chlorfenvinphose.

In vitro studies were performed with the goats using lactose solution at 10.8mg per ml. whereas *in vivo* studies were performed with the infected cattle, 2 of which were infused with lactose at 0.50 g per kg of body weight, three times a day at 4 hours intervals for 4 days, while the other 2 remained uninfused. All experimental animals were sacrificed immediately after their first peaks of parasitaemias.

3.2.2 Samples Collections and Preparations:

i. Ten (10) ml of blood was collected by jugular venipuncture from each of the infected and non-infectd goats and from the infected, lactose infused and infected non-infused cattle. The blood samples were

transferred into vacutainers with ethylene diamino tetra-acetic acid (EDTA) as anticoagulant. The blood was mixed with phosphate buffer pH 7 and centrifuged at 2500 revolutions per minute (rpm) at 2°C for 5 minutes (Beckman Model J-21B) centrifuge. The supernatant was discarded and the sedimented erythrocytes were resuspended in phsophate buffer and re-centrifuged. The centrifugation step was repeated until the cell pellets were clear. The packed cell volume(PCV) and haemoglobin concentration(Hb) for all the animals(goats and cattle), infected, infected-lactose infused and the control were determined using haematocrit method(Table 3 and Appendix III).

ii. One gram of the liver from each goat and cattle was used. The liver samples were perfused with 10ml of warm isotonic 0.9% sodium chloride (NaCl) solution and 5ml of 0.2% (0.4g/200ml) pronase E (Sigma) in Hank's balance salt solution. The liver was excised, cut into small pieces in the petri dishes and incubated at 37°C in 15ml of the pronase E solution with continuous gentle shaking for 20 minutes. The cell suspension was filtered into plastic test tubes through nylon gauze. The filtrate was centrifuged at 2500 rpm at 2°C for 5 minutes (BECKMAN Model J-21 B centrifuge). The supernatant was discarded, the cell pellets (Kupffer cells) were gently resuspended in cold Hank's balanced salt solution.

The resuspended cell pellets were recentrifuged at 2500 rpm at 2°C for 5 minutes. The centrifugation step was repeated until the cell pellets were clear. The cell pellets (Kupffer cells) were finally resuspended in medium 199E (Sigma) mixed with 10% (0.3g/3ml) Bovine calf serum (Randox) in plastic test tubes in the presence or absence of 10.8mg/ml Lactose in case

of goats, for *in vitro* study. Lactose infusion replaced this latter step in the case of cattle. The cells were incubated at 37°C for 18 hours in humidified air containing 5% CO_2 in the presence of desialylated erythrocytes (from *T. vivax* infected goats and cattle) or normal erythrocytes of uninfected goats and cattle respectively, for erythrocyte macrophage (Kupffer cell) interaction. After the erythrocyte-macrophage interaction, smears of the cells were made on clean glass slides. The smears were allowed to dry at room temperature, fixed in methanol for 5 minutes and stained with Giemsa stain for 30 minutes. The slides were allowed to dry and were examined under the Zeiss microscope (X 40 Objective).

For quantification, the suggested method by Schauer *et al.* (1984) was used. One hundred Kupffer cells (macrophages) were counted and percentage macrophages with binding and erythrophagocytosis, percentage macrophages with phagocytosis but without binding, percentage binding (rosette) and percentage macrophages that have neither binding nor erythrophagocytosis were determined and the data was recorded.

3.3.0 RESULT

3.3.1 A. Without Lactose:-

i. *T. vivax* infected goats.

Effects of interactions between red cells and isolated liver kupffer cells from *T. vivax* infected goats are presented (Table 1).

Percentage of Kupffer cells binding to and with phagocytosed red cells was between 12 and 19%, while percentage of Kupffer cells with phagocytosed red cells but without surface bound red cells was between 7 and 12%. Percentage of Kupffer cells with red cells bound to their surface but without phagocytosed red cells were between 18

and 40%. Kupffer cells with red cells neither bound to their surface nor phagocytosed was between 29 and 63%.

ii. Uninfected goats.

Interactions of normal erythrocytes with Kupffer cells (from uninfected goats) in the presence of bovine calf serum showed decreased binding and erythrophagocytosis. Percentage of Kupffer cells with bound red cells and erythrophagocytosis was between 1 and 3%, Kupffer cells showing erythrophagocytosis only, without bound red cells were 2% and those with bound red cells without erythrophagocytosis were between 10 and 29%. Kupffer cells with red cells neither bound nor phagocytosed were between 68 and 85%(Table 1).

iii. *T. vivax* infected and non-lactose infused cattle.

Percentage of Kupffer cells bound to erythrocytes and with erythrophagocytosis was between 13 and 14%, while percentage kupffer cells with phagocytosis but without bound red cells was between 4 and 9%. Kupffer cells with red cells bound to their surfaces only, accounted for 36 to 45%. However, Kupffer cells with red cells neither bound nor phagocytosed accounted for 38 to 41% (figs. 1 and 2, table 2).

iv. Uninfected cattle.

Normal erythrocytes interacted with Kupffer cells in the presence of bovine calf serum also showed markedly decreased binding and erythrophagocytosis as shown on Table 2 no.5.

3.3.2 B. Effects of Lactose:-

Interactions of the desialylated erythrocytes with Kupffer cells and lactose as inhibitor (*in vitro* in goats and *in vivo* in cattle) in the presence of bovine calf serum showed remarkable inhibition of binding and erythrophagocytosis.

i. *T. vivax* infected goats.

Only 2% of Kupffer cells showed bound red cells and erythrophagocytosis, while between 2 and 4% of Kupffer cells showed erythrophagocytosis without red cells bound to their surfaces. Kupffer cells with bound red cells, but without erythrophagocytosis accounted for between 4 and 8%, while 88 and 90% showed neither binding nor erythrophagocytosis (Table 1).

ii. Uninfected goats:

In another experiment, with uninfected goats' normal erythrocytes interaction with Kupffer cells in lactose and 10% bovine calf serum, only 0% was observed for binding and erythrophagocytosis and only 1% for Kupffer cells with erythrophagocytosis but without bound red cells. Binding of red cells to Kupffer cells alone, without erythrophagocytosis accounted for 2 to 3%, while neither erythrophagocytosis nor bound red cells on Kupffer cells accounted for 96 to 97% (Table 1).

iii. *T. vivax* infected and lactose infused cattle.

Kupffer cells with bound red cells and erythrophagocytosis were between 0 and 1%. One percent of Kupffer cells showed erythrophagocytosis without bound red cells, while 10 to 34% of Kupffer cells had bound red cells without erythrophagocytosis.

Neither erythrophagocytosis nor binding of red cells to Kupffer cells accounted for 64 to 90% (Table 2, Nos. 3 & 4).

All three processes, namely, binding and erythrophagocytosis, phagocytosis without binding and binding without phagocytosis, which eventually terminate in sequestration of desialylated erythrocytes by the expanded mononuclear phagocytic (reticuloendothelial) system, account for 71% and 62% in *T. vivax* infected goats and cattle respectively (Table 1 and 2).

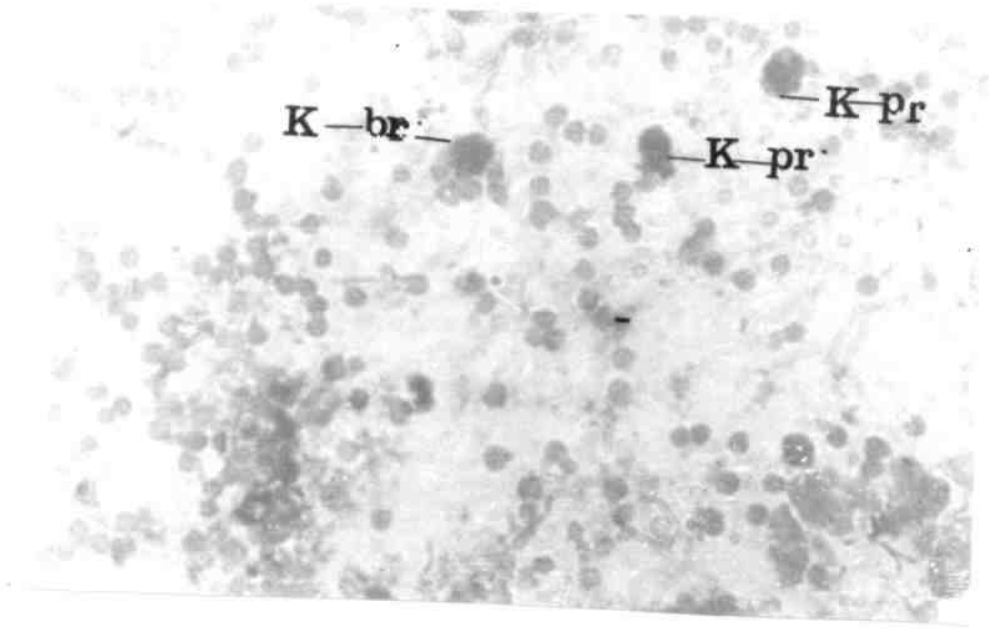


Figure 1: Binding and phagocytosis of homologous desialylated erythrocytes from T. vivax infected, non lactose infused cattle by Kupffer cells isolated from liver of the same cattle. K-br(Kupffer cell with bound erythrocytes); K-pr (phagocytosed erythrocytes). Giemsa stain X400.

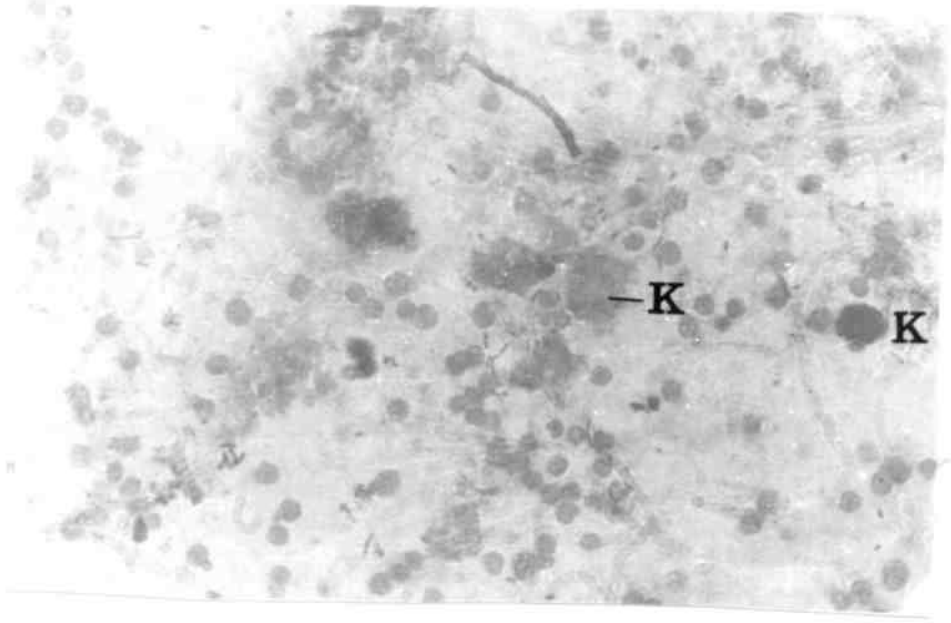


Figure 2: Reduced binding and phagocytosis of desialylated erythrocytes by Kupffer cells from *T. vivax* infected, lactose infused cattle (K). Giemsa stain X 400.

TABLE 1: BINDING AND PHAGOCYTOSIS OF NORMAL AND DESIALYLATED RED CELLS FROM CAPRINE *T. vivax* INFECTION IN THE ABSENCE OR PRESENCE OF LACTOSE (UNIT).

Case no.	Number of Phagocyti-sed RBC/*KPC	Treatment	Binding and phagocytosis (%)	Phagocytosis only (%)	Binding only(%)	No binding No phagcy-tosis (%)
1(a)	1-2	Desialylated RBC +Kupffer cells +1.0mlbovine calf serum.	19	12	40	29
1(b)	0-1	Normal RBC + Kupffer cells +1.0ml bovine calf serum.	1	2	29	68
1(c)	0-1	Desialylated RBC + Kupffer cells + 5.0ml lactose + 1.0ml bovine calf serum.	2	2	8	88
1(d)	0-1	Normal RBC + Kupffer cells + 5.0ml lactose + 1.0ml bovine calf serum	0	1	2	97
2(a)	1-2	Desialylated RBC + Kupffer cells + 1.0ml bovine calf serum.	12	7	18	63
2(b)	0-1	Normal RBC + Kupffer cells + 1.0ml bovine calf serum.	3	2	10	85
2(c)	0-1	Desialylated RBC + Kupffer cells + 5.0ml lactose + 1.0ml bovine calf serum.	2	4	4	90
2(d)	0-1	Normal RBC + Kupffer cells + 5.0 ml lactose + 1.0ml bovine calf serum	0	0	3	97

* KPC = KUPFFER CEL

TABLE 2: BINDING AND PHAGOCYTOSIS OF NORMAL AND DESIALYLATED RED CELLS FROM BOVINE *T. Vivax* INFECTION IN THE ABSENCE OR PRESENCE OF LACTOSE(UNIT)

Case No.	No.of RBC/*KPC	Treatment	Binding and phagocytosis (%)	Phagocytosis only (%)	Binding only(%)	No binding No phagocytosis (%)
1.	RBC 1-2	Desialylated RBC + Kupffer cells + 1.0ml bovine calf serum	13	4	45	38
2.	RBC 1-3	Desialylated RBC + Kupffer cells + 1.0ml bovine calf serum.	14	9	36	41
3.	RBC 0-1	Desialylated RBC + Kupffer cells +infused lactose + 1.0ml bovine calf serum.	1	1	34	64
4.	RBC 0-1	Desialylated RBC + Kupffer cells +infused lactose + 1.0ml bovine calf serum.	0	0	10	90
5.	RBC 0-1	Normal RBC + Kupffer cells + 1.0 ml bovine calf serum.	0	0	5	95

*KPC = KUPFFER CELL

3.4.0 Discussion

This study showed that Kupffer cells (hepatic macrophages) either bound alone or bound and phagocytosed homologous desialylated erythrocytes, both of which were isolated from *T. vivax* infected goats and cattle respectively, during the first peaks of parasitaemias and periods of development of anaemia. Recognition and binding of red cells or foreign structures had been recognised as the first step of red cell and macrophage interaction leading to phagocytosis (Kuster and Schauer, 1981; Mohr, *et al.* 1987). Thus it is reasonable to presume that all the processes of binding and phagocytosis, phagocytosis without binding and binding without phagocytosis observed in this study, are one and the same process terminating as sequestration of desialylated red cells by the expanded mononuclear phagocytic (reticulo-endothelial) system. As a dynamic on-going process, each step or phase expectedly occurs at any sampling time. Therefore, these processes culminating in phagocytosis of desialylated red cells, totalling upto 71% in *T. vivax* infected goats against 32% in normal goats' erythrocytes (Table 1) and up to 62% in *T. vivax* infected cattle against 5% in normal cattle erythrocytes (Table 2) are very significant. *T. vivax* produces sialidase, the activity of which is proportional to the number of parasites (Esievo, 1979; 1983). The pathogenesis of the anaemia in *T. vivax* infected cattle, was linked to a first step removal of erythrocytes surface sialic acid (Esievo *et al.* 1982). Only recently, it was shown in *T. vivax* infected cattle, that cleavage of erythrocytes sialic acids exposed penultimate B-galactosyl residues on their erythrocyte membranes (Omage - Personal communications).

Bacterial sialidases, specificaly, those of *Clostridium Perfringens* and *Vibrio cholera* were used to desialylate erythrocytes of rabbits and cattle and thus demask their surface B-galactosyl residues; the binding of the latter by *B*-galactosyl-specific lectin on the peritoneal and liver

(Kupffer cell) macrophages was shown to be involved in their subsequent phagocytosis, as the mechanism of sequestration of desialylated erythrocytes *in vivo* (Jancik *et al.*, 1978; and Kuster and Schauer, 1981; Muller *et al.*, 1981; Muller and Schauer, 1983). With the production of sialidase by *T. vivax* infected cattle (Esievo *et al.*, 1982), and the exposure of galactosyl residues on erythrocytes of cattle infected with *T. vivax* (Omage personal communications) the finding in this present study of enhanced Kupffer cells activities of binding and phagocytosis of homologous desialylated erythrocytes from *T. vivax* infected goats and cattle very strongly suggest that similar lectin based erythrophagocytosis is operative, partly, very early in ruminant trypanosomiasis, as a mechanism of sequestration of desialylated erythrocytes by the expanded mononuclear phagocytic (reticulo-endothelial) system. It sounds reasonable to hypothesise that very early in acute *T. vivax* infection in ruminants, when an immune response has not been fully operative in the infected host, *T. vivax* sialidase cleaves off erythrocytes surface sialic acids, exposes the penultimate *B*-galactsyl residues on the erythrocyte membranes, this initiates an activated interaction between the kupffer cells' and other macrophage surface lectin binding onto the *B*-galactose of the desialylated erythrocytes for subsequent phagocytosis and sequestration.

Only the amount of released sialic acids is the decisive factor in the binding and phagocytosis of sialidase-treated erythrocytes (Muller *et al.*, 1983a; Muller, *et al.*, 1983b), by a mechanism independent of opsonins but as a function of the number of the desialylated erythrocytes and the binding strength of the macrophages (Muller *et al.*, 1983b). This gives credence to the observation by Omage (personal communication) of the direct relationships between the number of rosettes formation, free serum sialic acids and parasitaemia in *T. vivax* infected cattle; the parasitaemia being proportional to the activity of the sialidase produced (Esievo, 1979;

1983). Therefore, the observed values of 71% and 62% for the processes leading to sequestration of desialylated erythrocytes in *T. vivax* infected goats and cattle respectively, in this study, are indirect measurements of the amount of sialic acids released from the hosts erythrocytes and partly, the units of sialidase activity of the trypanosome parasitaemias in the respective hosts.

Remarkable inhibition of binding and phagocytosis of homologous desialylated erythrocytes by isolated kupffer cells from the *T. vivax* infected goats and cattle was achieved by prior *in vitro* interactions with lactose in goats or *in vivo* lactose infusion in cattle. This observation agrees with the report of Muller *et al.* (1981) in which lactose resulted in higher percent inhibition by preventing desialylated erythrocyte – macrophage interactions, a process whereby the lactose, with its galactose end, binds competitively to Kupffer cells surface lectin, preventing the latter from recognising and binding to penultimate *B*-galactosyl residues exposed on the desialylated erythrocytes membranes. The latter was confirmed, similarly in *T. vivax* infected cattle, using rat peritoneal macrophages (Omage – personal communication).

This report appears to be the first, incriminating macrophage, specifically Kupffer cells surface lectin involvement in the erythrophagocytosis occurring very early in acute ruminant *T. vivax* infection and strongly supports lactose chemotherapeutically as a base to trypanocidal agents.

3.5.0 SUMMARY

In the present study, binding and subsequent erythrophagocytosis was inhibited in ruminants (goats and cattle) by lactose, both *in vitro* and *in vivo*. This agreed with earlier experimental report in rats by Kolb and Kolb-Bachofen (1978) and Muller *et al.* (1981). The authors reported that,

adherence (binding) was mediated by *B*-glycosidically bound galactose on desialylated erythrocytes to the macrophage (Kupffer cell) surface lectin. Both the binding and erythrophagocytosis were inhibited in rats by lactose (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Kuster and Schauer, 1981) and in ruminants in the present study. The most important beneficial inhibitory effect of lactose on binding and subsequent erythrophagocytosis, was the amelioration of the anaemia in ruminant trypanosomiasis observed in the present study.

CHAPTER FOUR

4.0 THE EFFECT OF LACTOSE INFUSION ON THE PATHOLOGICAL MANIFESTATIONS OF *Trypanosoma vivax* INFECTION IN CATTLE

4.1.0 INTRODUCTION

Despite the fact that *T. vivax* infection had long constituted a major component in poor production and losses in cattle in West Africa (Edward *et al.*, 1956), the pathogenesis and pathology of trypanosomiasis in cattle have remained the subject of intensive investigations (Saror, 1980). A principal pathological feature of trypanosomiasis is anaemia. Experimental evidence indicates that the anaemia is haemolytic in nature and that the haemolysis occurs principally extravascularly in expanded mononuclear phagocytic system(Fiennes, 1970; Naylor, 1971; Losos and Ikede, 1972; Saror, 1975; Van Den Ingh *et al.*, 1976). The phenomenon of erythrophagocytosis as a contributing factor to the anaemia is well documented (Mackenzie and Kruickshank, 1973; Esievo *et al.*, 1982; Esievo and Saror, 1991). Binding between demasked erythrocyte surface *B*-galactosyl residues, to the lectin on the macrophage cell membrane is necessary for erythrophagocytosis (Muller *et al.*, 1981; Muller and Schauer, 1983). The binding can be inhibited by *B*-D-galactosyl containing glycoproteins including lactose (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Muller and Schauer, 1983) and such inhibition has been experimentally produced with lactose in animal trypanosomiasis (see Chapter III). Recent information indicates that lactose has the potential of serving as a base for trypanocidal agents in eradication or control of animal trypanosomiasis (see Chapters III and IV). This project was therefore designed to re-examine the role of lactose infusion on the pathological manifestations of *T. vivax* infection in cattle.

4.2.0 MATERIALS AND METHODS

4.2.1 (a) The Parasite:

Trypanosoma vivax (Samaru stock) was obtained from a cow with natural infection. Two millilitre of the infected blood from the cow was used to infect a goat that served as donor animal. At the first peak of parasitaemia the blood of the donor goat was used to infect the calves. experimentally.

4.2.2 (b) The Experimental Animals:

Twelve Sokoto gudali calves, aged between 12 and 18 months and between 80 and 90kg body weight were purchased from Talatan-Mafara, Sokoto State, Nigeria, an area known to be tsetse-fly free. On arrival in Zaria, they were dewormed with thiabendazole, vaccinated against contagious bovine pleuropneumonia, rinderpest, blackquarter and anthrax. They were sprayed with chlorfenvinphose (Pfizona, Pfizer). The animals were kept in a pen, fed with diet supplemented with a mixture of wheat offal and hay. Their blood was taken and screened for haemoparasites. Water and salt licks were provided *ad libitum*.

4.2.3 (c) Infection of the Experimental Animals:

The animals were divided into four animals per group and four control animals were also maintained. Amongst the infected-non-lactose infused calves 2 were sacrificed; also amongst the infected-lactose infused calves 2 were sacrificed. One of the control calves was also sacrificed for detailed gross and histopathological studies.

4.2.4 (d) The calves were housed separately:

Except for the calves that served as control, the calves in each group were infected with 11 x 10^6 trypanosomes intravenously with sterile syringe and needle. The level of parasitaemia was monitored daily by HCT, wet mount and haemocytometer techniques.

4.2.5 (e) Preparation of Lactose Solution for Infusion:

A 50% solution of lactose in physiological saline (0.9% Nacl) was prepared and placed in an injection bottle, plugged, autoclaved at 15lbs presure for 5 minutes and allowed to returned to room temperature. Normal saline as a solvent was preferred to prevent any minimal systemic haemolysis. Approximately, 80 to 90ml of the lactose solution was administered into four of the infected calves at the peak of the parasitaemia(3-6 days), depending on body weight. It was administered at the rate of 0.5g lactose per kg body weight, three times daily at four hours intervals, intravenously, with sterile syringes and needles at approximately 18ml/ minute (Umar, personal communication). The lactose infusion was done for four days. The lactose solution was not administered into the remaining four infected calves. The level of parasitaemia was monitored and at the end of 5 days all the calves were sacrificed. The carcasses were examined grossly and the gross lesions were recorded.

4.2.6 (f) Histopathology:

Tissue sections of the liver, kidney, spleen, prehepatic and messenteric lymph nodes, lung, heart, intestine and brain samples were fixed in 10% buffered formalin (PH 7.4) for at least 48 hours. The tissues were processed using technicon and sectioned at 5 to 6um. The tissue sections were mounted on clean glass slides in the presence of egg albumin, dried at room temperature and stained with hematoxyline and eosin. The

stained tissue was cover-sliped and dried in the oven. The slides were examined microscopically (Olympus x 40 objective). The histopathological findings were recorded.

4.3.0 RESULTS

4.3.1 (i) Infected-non-lactose infused calves.

(a) Gross lesions:

The gross lesions observed in the carcasses of the *Trypanosoma vivax* infected-non-lactose infused calves include pale mucous membranes (severe anaemia)(Table 3), rough hair coat, emaciation, serous atrophy of fat, hepatomegally, splenomegaly and enlarged oedematous lymph nodes.

(b) Microscopic lesions:

In the liver, there was diffuse areas of necrosis of the hepatocytes, erythrophagia by the Kupffer cells, dilated sinusoids(at the portal area) and mononuclear cellular infiltration into the necrotized areas (Fig. 3).

In the spleen and lymph node, there were lymphocyte proliferation(active follicles), plasma cells, haemosiderosis and haemosiderin laden macrophages (Fig.4)

The kidney had hypercellular glomeruli and adhesion of the parietal layer of the glomeruli to the Bowman's capsule, necrosis of the proximal, distal and collecting tubules, periglomerular and inter-tubular mononuclear cellular infiltration into the necrotized areas (Fig.5).

The heart had minimal myocarditis. The brain, lung, and the intestine had no singnificant histopathlogical lesions.

4.3.2 (ii) Infected-lactose-infused calves:

(a) Gross lesions:

(i) Pre-lactose infusion: The gross lesions in the carcases of *Trypanosoma vivax* infected, lactose-infused calves were pale mucous membranes (severe anaemia)(Table 3) and emaciation.

(ii) Post-lactose infusion: The mucous membranes were slightly pale (slightly anaemic)(Table 3). The carcasses were relatively emaciated at the time of necropsy and there were no significant gross lesions in other organs.

(b) Microscopically:

There was fatty degeneration of the hepatocytes and scanty erythrophagia by the Kupffer cells at the centrilobular area, compared to that of infected-non-lactose infused calves (Figs. 3 and 6). There was less dense lymphoid hyperplasia in the spleen(Fig. 7). The glomeruli were hypercellular and there was no adhesion. The Bowmans space was wide and there was no necrosis of the renal tubules(Fig. 8). There was no significant histopathological lesions in the heart, lung, brain, and intestine.

4.3.3 The Control Calf:

a) Gross lesions: There was no observable gross lesions in the carcass.

b) Microscopically: The liver, kidney, spleen(Figures 9, 10 and 11), lymph node , lung, heart, brain, and intestine did not show significant histopathological lesions.

TABLE 3: PROFILES OF MEAN PCV FOR ALL GROUPS.

Days Post Infectiion	Mean PCV (%) Infected Infused group	Infected Uninfused group	Uninfected Uninfused (Control) group
0	25.5	20.0	25.0
1	25.5	20.5	24.5
2	25.5	21.0	24.5
3	25.0	20.5	24.5
4	25.5	20.0	25.0
5	24.5	18.5	25.0
*6	22.5	18.0	25.5
7	24.0	16.5	25.0
8	20.0	13.0	25.0
9	22.5	16.5	25.0
**10	21.0	16.0	25.0
11	18.5	16.5	26.0
12	19.5	20.0	26.0
13	18.0	17.0	25.0

* The day when lactose infusion started.

** The last day of lactose infusion.

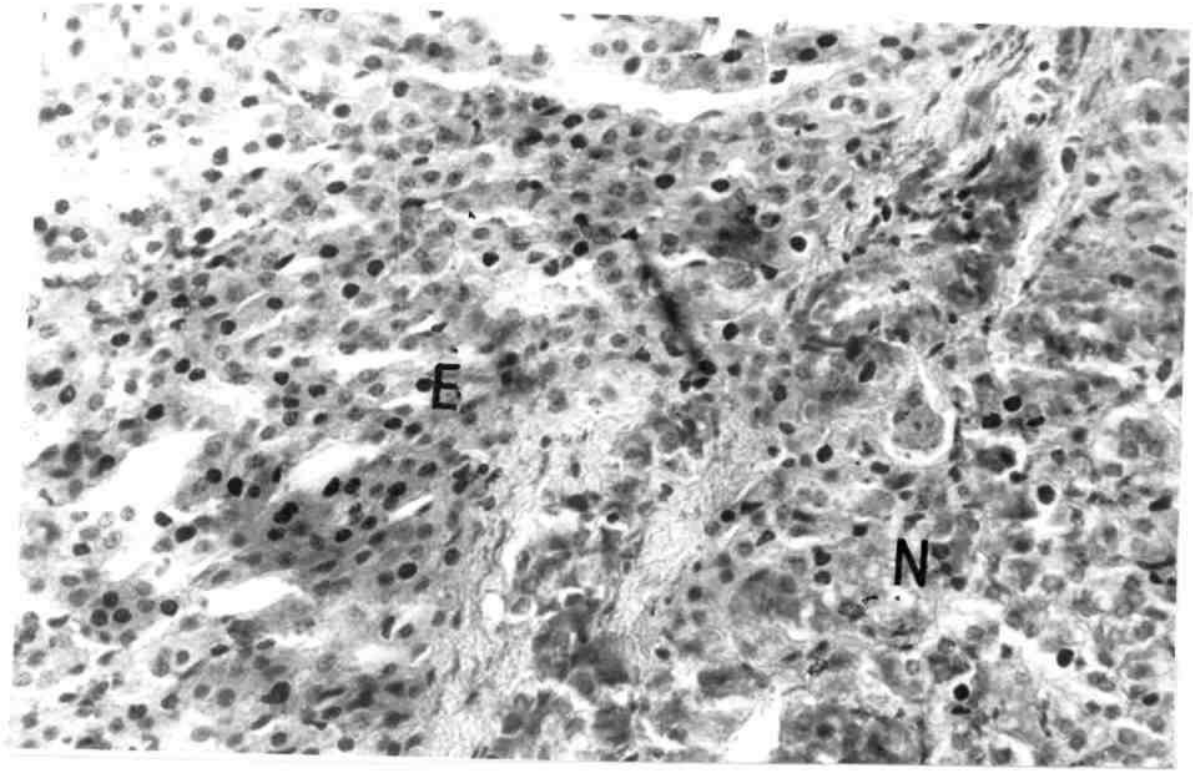


Figure 3: Histopathological section from the liver of a *T. vivax* infected calf. Note necrosis of the hepatocytes(N), erythrophagia(E) and dilated sinusoids at the portal area. H & E X400.

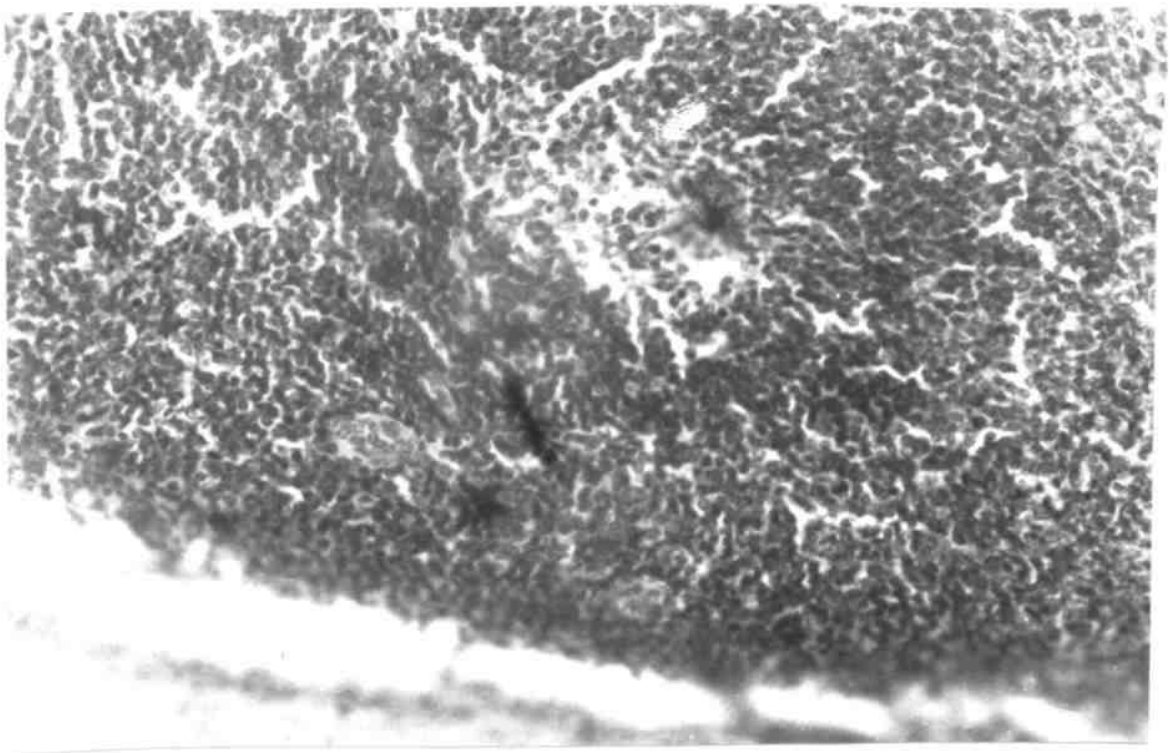


Figure 4: Histopathological section from the spleen of a *T. vivax* infected calf. Note the lymphocytes proliferation (active follicles) in the spleen. H & E X400.

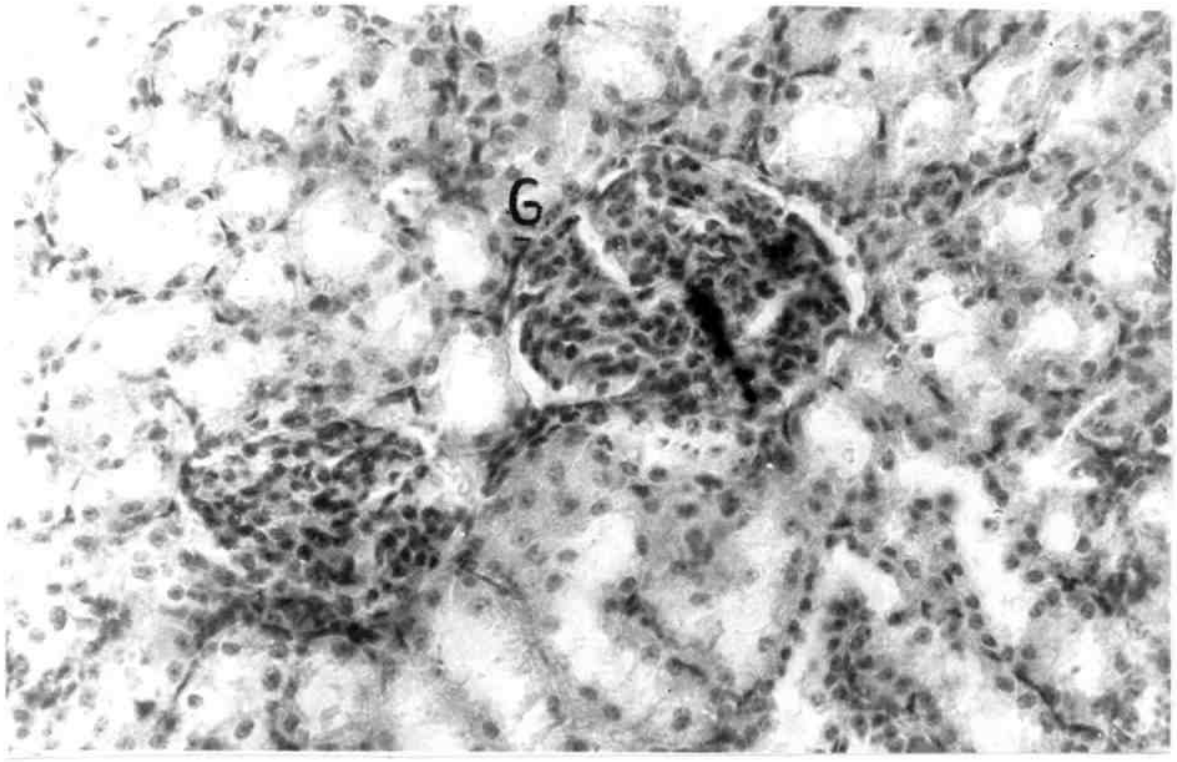


Figure 5: Histopathological section from the kidney of a *T. vivax* infected calf. Note hypercellular glomeruli, adhesion of the parietal layer of the glomeruli to the Bowman's capsule (G), necrosis of the renal tubules and periglomerular and inter-tubular mononuclear cellular infiltration. H & E X400.

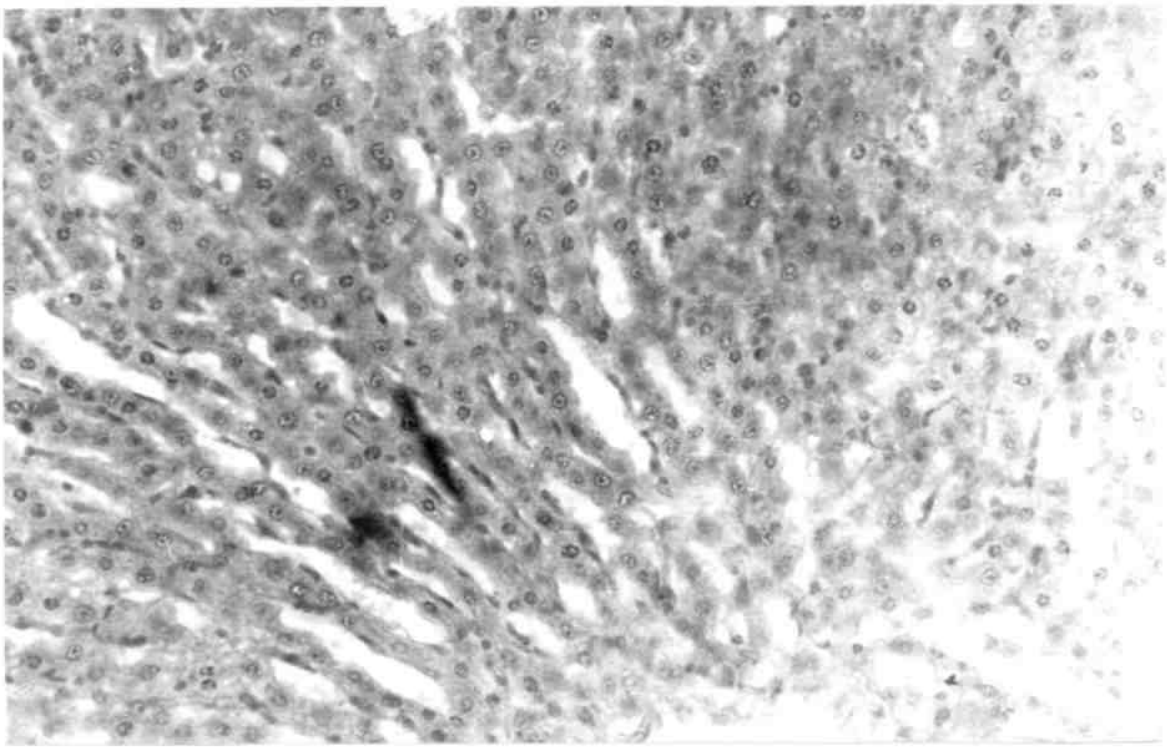


Figure 6: Histopathological section from the liver of a *T. vivax* infected, lactose infused calf. Note fatty degeneration of the hepatocytes and scanty erythrophagia at the centrilobular area. H & E X400.

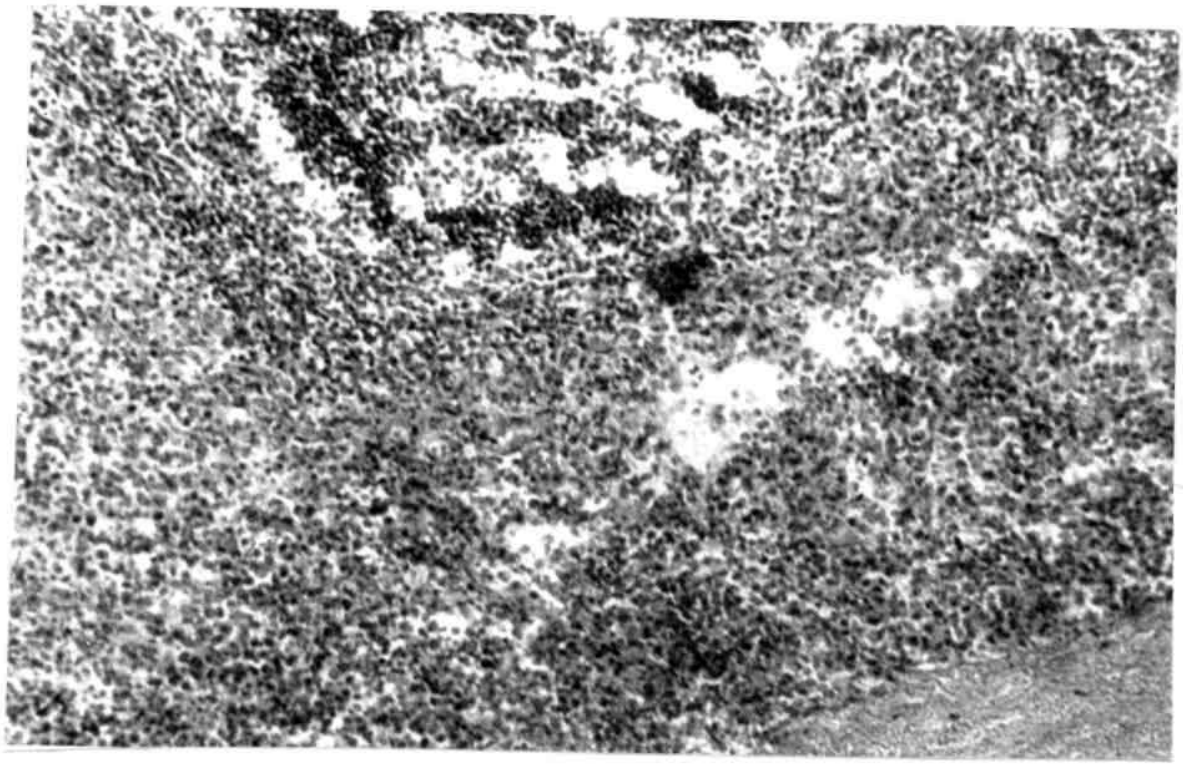


Figure 7: Histopathological section from the spleen of a *T. vivax* infected, lactose infused calf. Note the less dense lymphoid hyperplasia in the spleen. H & E X400.

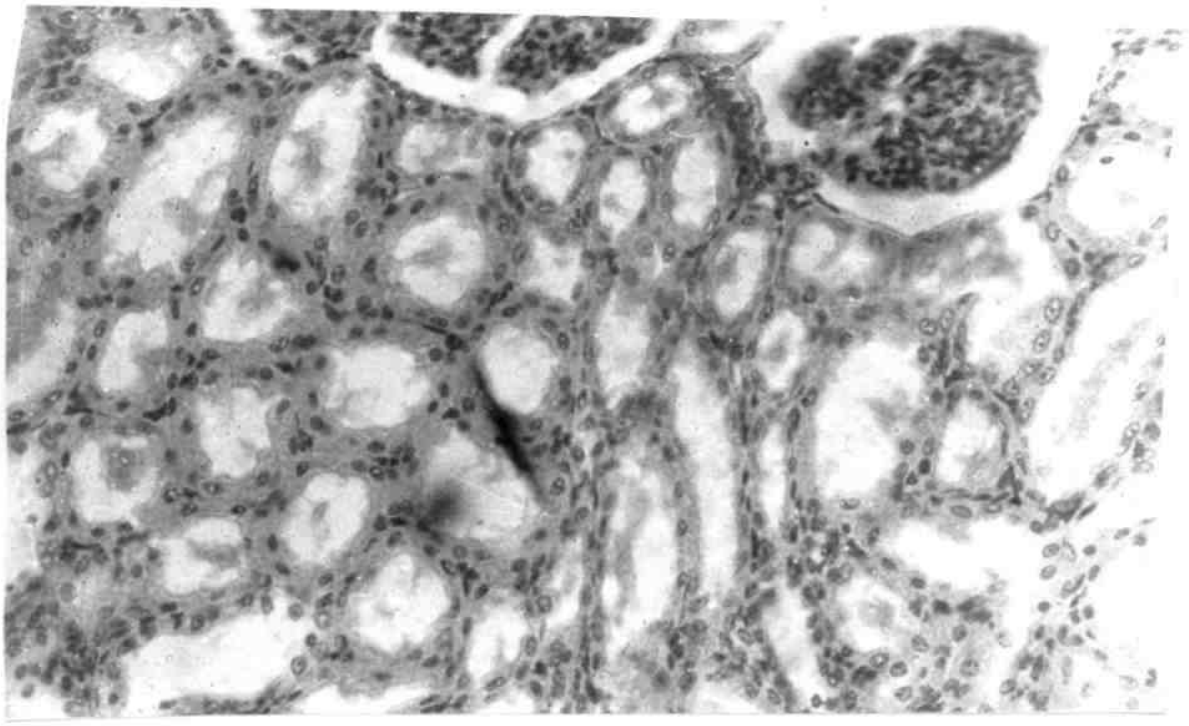


Figure 8: Histopathological section from the kidney of a *T. vivax* infected, lactose infused calf. Note the wide Bowman's space with no adhesion of the parietal layer of the glomeruli to the Bowman's capsule. Also note the absence of renal tubular necrosis. H & E X400

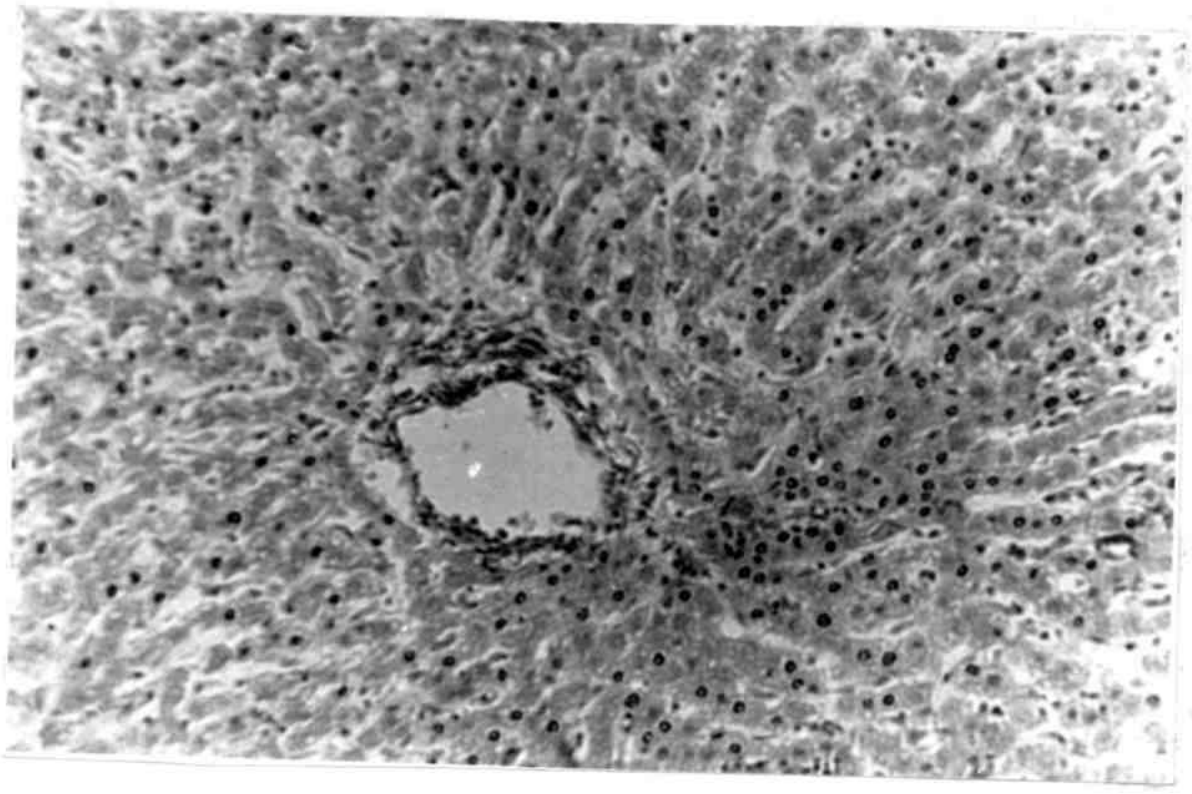


Figure 9: Histopathological section from the liver of the control. No significant histopathological lesions. H & E X400.

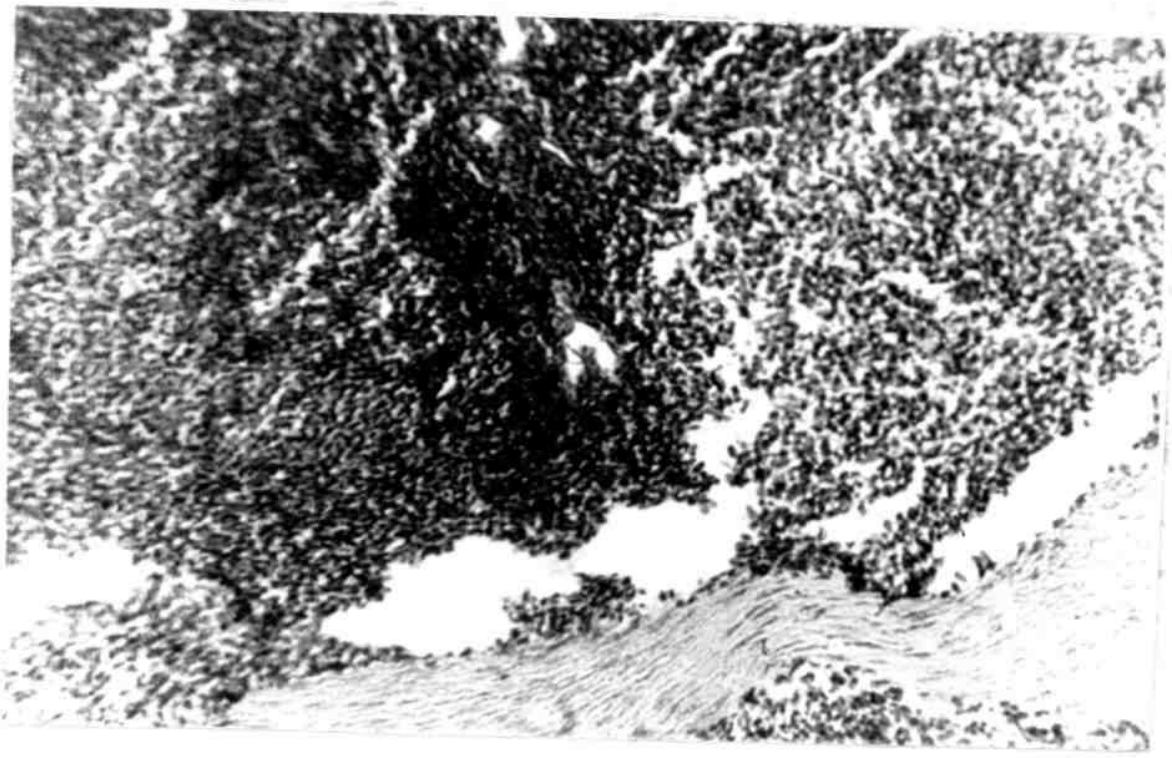


Figure 10: Histopathological section from the spleen of the control No significant histopathological lesions. H & E X400.

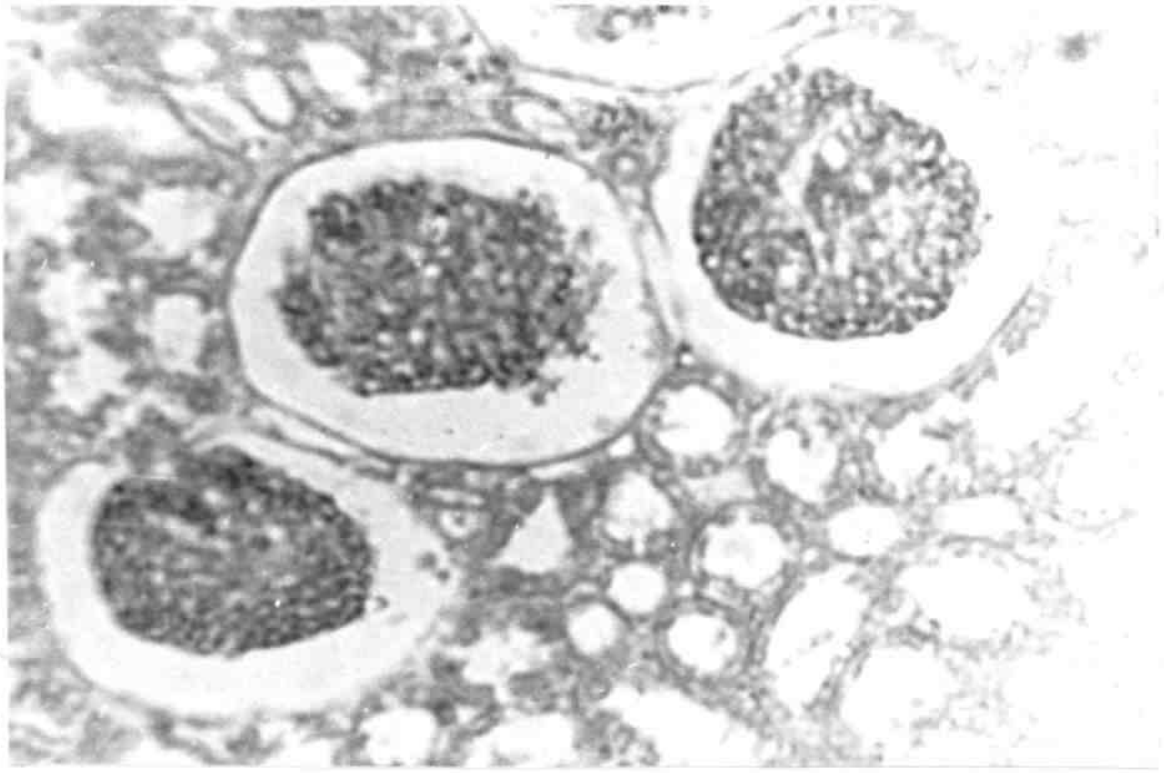


Figure 11: Histopathological section from the kidney of the control. No significant histopathological lesions. H & E X400.

4.4 DISCUSSION:

The predominant gross pathological features in trypanosomiasis include anaemia, rough hair coat, emaciation, serous atrophy of the coronary fat, hepatomegaly, splenomegaly and enlarged oedematous lymph nodes(Fiennes, 1970; Naylor, 1971; Isoun and Esuruoso, 1972; Losos and Ikede, 1972; Jenning *et al.*, 1974; Murray *et al.*, 1974; Van Den Ingh *et al.*, 1976; Anosa and Isoun, 1976; Saror, 1980; Esievo *et al.*, 1982). Severe anaemia, rough hair coat, emaciation, and lymphadenopathy were observed in all the infected calves and at pre-lactose infusion stage after establishment of the disease in the present study. In addition other observed gross lesions in the infected non lactose infused calves were hepatomegaly, splenomegaly, lymphadenopathy and serous atrophy of fat. Following lactose infusion, the anaemia was ameliorated, it became mild and the infected lactose infused calves then became only slightly emaciated. The liver, spleen, lymph node, and kidney had no observable gross lesions, so also other organs of the carcasses of the infected lactose infused calves, an observation that was not reported earlier.

The microscopic features commonly associated with trypanosomiasis include active follicles with enlarged germinal centres in the lymph node and spleen. Hyperplastic lymphocytes, haemosiderin laden macrophages in the spleen, lymph node, and liver, necrosis and erythrophagocytosis by Kupffer cells in the liver. Hypercellular glomeruli, necrosis of the renal tubules, in some cases dilated proximal tubules with cuboidal epithelial cells, oedema in the alveoli, myositis and myocarditis in some cases, mononuclear cellular infiltration(Fiennes, 1970; Naylor, 1971; Isoun and Esuruoso, 1972; Losos and Ikede, 1972; Anosa and Isoun, 1976; Van Den Ingh *et al.*, 1976; Saror, 1980).

In the present study, there was necrosis of the hepatocytes, erythrophagia by the Kupffer cells similar to previous report by Van Den

Ingh *et al.*(1976) and dilated sinusoidal spaces in the infected-non-lactose infused calves. These observed histopathological lesions may indicate that there was interference with the normal cellular and biochemical changes resulting in prevention of the normal physiological function of the liver such as biliary secretion and detoxification. In contrast, the liver of the infected-lactose infused calves had fatty degeneration of the hepatocytes, and scanty erythrophagia(Fig. 3 and 6). These observations compare favourably with that of the control uninfected calf, suggesting that the lactose infusion into *T. vivax* infected cattle may be preventing the tissue and cellular damage as manifested histopathologically in the organs associated with the infection. These may lead to near normal, in the restoration of the morphological structure and normal physiological function of the organ.

The observed hypercellular glomeruli in the kidney of the infected calves was similar to that of earlier report by Valli and Forsberg(1979) and in addition, in the present study there was adhesion of the parietal layer of the glomeruli to the Bowman's capsule, necrosis of the renal tubules with mononuclear cellular infiltration. However, in the infected lactose infused calves the Bowman's space was dilated and there was no adhesion of the glomeruli to the Bowman's capsule or renal tubular necrosis(Fig. 5 and 9). It would appear that the role of lactose in the prevention of microscopic damages in the liver as stated above also applies to the kidney. This implies that, it prevented destruction of the normal morphology of the tissues and cells of the liver, spleen and kidney to near normal morphology.

In this study there was proliferation of hyperplastic lymphocytes, plasma cells, haemosiderin-laden macrophages and very active splenic follicles in the infected non lactose infused calves. Similar report was made(Van Den Ingh *et al.*, 1976; Valli and Forsberg, 1979; Saror, 1980).

However, in contrast, the present study has shown that the lymphoid hyperplasia was less dense in the infected lactose infused calves(Fig. 4 and 7), further supporting the role of lactose in preventing or reducing microscopic damages during *T. vivax* infection in cattle.

It may be concluded that, the inhibitory effect of lactose on tissue and cellular injury and erythrophagocytosis by Kupffer cells as shown in the infected lactose infused calves(Fig. 3 and 6) can result in the following: Amelioration of anaemia, prevention of destruction of the normal morphology of the tissues and cells of the liver, spleen and kidney to near normal in trypanosomiasis. This, implies that probably the normal physiological functions of these organs, such as detoxification of toxic substances by the liver, excretion of such toxic substances by the kidney of the animal suffering from trypanosomiasis may be restored by concurrent lactose infusion during chemotherapy of trypanosomiasis or by using lactose as a base for trypanocidal agents.

4.5.0 SUMMARY:

The amelioration of the anaemia (Ibrahim *et al.*, 1996) and preservation of the cellular morphology of the various organs, as observed in the present study are good indicators. Prior to this investigation there was scanty information if any, on the inhibitory role of lactose on the anaemia and prevention of tissue damage in ruminant trypanosomiasis. Previous reports were on rats(Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Kuster and Schauer, 1981) and not in relation to trypanosomiasis. The results of this study indicates that lactose infusion influences positively the course of trypanosomiasis in cattle. It appears that incorporation of lactose in the chemotherapy of trypanosomiasis may have benefitial effects. The results of this study suggests incorporation of lactose in chemotherapy of trypanosomiasis in ruminants.

CHAPTER FIVE

5.0 IN VITRO STUDIES ON INTERACTION OF COMMERCIAL NEURAMINIDASE-TREATED AND UNTREATED ERYTHROCYTES WITH KUPFFER CELLS IN THE PRESENCE OR ABSENCE OF LACTOSE.

5.1.0 INTRODUCTION

Reduction of erythrocytes life-span and erythrophagocytosis are constant contributing factors in the etiology of the anaemia of cattle trypanosomiasis (Mackenzie and Cruickshan, 1973; Dargie et al., 1979). Loss of erythrocyte surface sialic acids during the peak of parasitaemia in trypanosomiasis and attendant highest production of trypanosome sialidase had been incriminated for the shortening of erythrocyte life-span and erythrophagocytosis (Esievo *et al.*, 1982).

The mechanisms involved in the destruction of desialylated erythrocytes by the liver (Kupffer cells) and peritoneal macrophages have been studied extensively. Kuster and Schauer (1981) reported that rat peritoneal macrophages bound homologous sialidase treated erythrocytes via a *B*-D-galactose-specific lectin on the macrophage surface. The *B*-galactosyl-specific lectin seems to be involved in the mechanism of sequestration of desialylated erythrocytes *in vivo*. Binding via demasked erythrocyte surface B-galactosyl residues to the lectin of the macrophage cell membrane is necessary for subsequent phagocytosis (Muller *et al.*, 1981; Muller and Schauer, 1983). Sialidase treatment exposes *B*-galactose residues of the erythrocyte membrane and interaction of these cells with a *B*-specific lectin on the liver and spleen macrophages (Jancik *et al.*, 1978) results in rapid sequestration of the sialidase-treated erythrocytes from the bloodstream (Muller *et al.*, 1981). The amount of released sialic acids is the decisive factor for the binding and phagocytosis of sialidase-treated erythrocytes (Muller *et al.*, 1983 a,b.). Binding and phagocytosis of sialidase-treated rat erythrocytes occurred by a mechanism independent of opsonin (Muller et al., 1983b) as involvement of immunoglobulins and complement

factors of the classical and alternative pathway in the engulfment process was excluded (Muller *et al.*, 1983 b). The desialylated erythrocytes bound to the *B*-galactose specific lectin on the marcrophage surface are phagocytosed as a function of their number and binding strenth to the macrophage (Muller *et al.*, 1983 b). Both binding and phagocytosis were inhibited by D-galactosyl compounds and other related sugars including lactose (Kolb and Kob-Bachofen, 1978; Muller *et al.*, 1981).

The binding of asialo-erythrocytes is not dependent on the metabolic state of Kupffer cells since clusters (rossets) were formed at 0^{0}C and at 37^{0}C, both living and dead cells were involved(Kolb and Kolb-Bachofen, 1978). The mechanism for binding and subsequent phagocytosis in the presence of serum, seems to operate similarly in the *in vitro* and in living animals (Kolb and Kolb-Bachofen, 1978).

This study reports that the cattle that were brought to the abbatoir were carrying subclinical infections in which the pathogens were capable of producing neuraminidase. Such pathogens are *Clostridium perferenges*, *Vibrio cholera* and trypanosomes. This observation rendered abbatoir cattle not to be suitable for experimental work that involves infection with *Trypanosoma vivax*.

5.2.0 MATERIALS AND METHODS

Livers from ten cattle were obtained from abbatoir and used for *in vitro* study on erythrophagocytosis and its inhibition. In the study, lactose 10.8mg/ml, was used as an inhibitor to asses the role of lectin on the surface of Kupffer cells in the bovine livers. Isolation of Kupffer cells, enzymic removal of sialic acids from erythrocytes using commercial neuraminidase (Sigma) were done according to the procedures described by Mills and Zucker-Franklin (1969) and Kuster and Schauer (1981).

5.2.1 Sample collection:-

i)a Ten millilitres of blood was collected using sterile 18G needle and 10.0ml syringe from the jugular vein of each of the abbatoir cattle used. The blood was transfered into labelled sample bottles containing EDTA 0.0014mg/ml as anticoagulant.

b. About 1gm of the liver was taken from each of the ten abbatoir cattle used after slaughtering. All samples were labelled, packed in cooled cotton wool with ice-pack and transported to clinical pathology laboratory, Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, for immediate analysis.

5.2.2 ii) Isolation of erythrocytes:-

Five millilitres of the blood sample from each animal was mixed with 5.0ml of barbital-buffered saline (140mm NaCl, 40mM EDTA, 4mM barbital 0.1% gelatine, pH 7.2), centrifuged (BECKMAN MODEL J-21B) in plastic test tubes, at 2500RPM for 5 minutes at 2^{oC}. The supernatant was discarded. The sedimented pellate was recentrifuged with phosphate buffered saline (PBS: 137mM NaCl, 2.mM KCl, 8.1mM Na2HPO4, 1.5mM KH_2PO_4, pH 7.2) at 2500RPM for 5 minutes at 2oC. The centrifugation step was repeated until the cell pellet was clear.

5.2.3. iii) Enzymic removal of sialic acid from the erythrocytes:-

Two millilitres of phosphate buffered saline was added to sedimented erythrocytes in each test tube and mixed gently. Sialidase (neuraminidase) 100mU from *Clostridium perferenges* (Sigma) was added to each of the test tubes except those intended to serve as normal erythrocytes for control. They were incubated at 37°C for 1 hr. At the end of the incubation period, the desialylated erythrocytes were used for erythrocyte-macrophage interaction.

5.2.4. iv) Isolation of Kupffer cells:-

One gram of the liver from each cattle was used. The liver was perfused with 10ml of warm isotinic 0.9% sodium chloride (NaCl) and 5ml of 0.2% (0.4g/200ml) pronase E (sigma) in Hank's balance salt solution. The liver was excised, cut into small pieces in the petri dishes and incubated at 37°C in 15ml of the pronase E solution with continous gentle shaking for 20mins. The cell suspension was filtered into plastic test tubes through nylon gauze. The filtrate was centrifuged at 2500RPM at 2oC for 5minutes The centrifugation step was repeated untill the cell pellet was clear. The cell pellet (Kupffer cells) was finally resuspended in medium 199E (Sigma) mixed with 10% (0.3g/3ml) Bovine calf serum (Randox) in plastic test tubes in the presence or absence of 10.8mg/ml lactose. The cells were incubated at 37oC for 18hr in humidified air containing 5% Co2 in the presence of desialylated or normal erythrocytes. This resulted in erythrocyte-macrophage (Kupffer cells) interaction and erythrophagocytosis. After the erythrocyte-macrophage interaction, smears of the cells were made on clean glass slides. The smears were allowed to dry at room temperature, fixed in methanol for 5 min. and stained with Giemsa stain for 30 minutes. The slides were allowed to dry and were examined under the Zeiss microscope (X40 objective).

For quantification, the suggested method by Schauer *et al.* (1984) and his personal communication was used. One hundred Kupffer cells (macrophages) were counted and percentage macrophages with binding and phagocytosis, phagocytosis but without binding, percentage binding (rosette) and percentage macrophages that have neither binding nor erythrophagocytosis were determined and the data was recorded.

5.3.0 RESULTS

5.3.1 i) Interaction between commercial neuraminidase - desialylated erythrocytes and Kupffer cells:-

The percentage of Kupffer cells with binding and erythrophagocytosis was 58 to 93%. The Kupffer cells with phagocytosed erythrocytes but no binding was 0 to 31%; while 4-23% of the Kupffer cells had binding without erythrophagocytosis. Zero to 4% of the Kupffer cells had neither binding nor erythrophagocytosis (Table 4).

5.3.2 ii) Interaction between untreated erythrocytes and Kupffer cells:-

Interaction of untreated erythrocytes with homologous Kupffer cells resulted in 12 to 65% binding and subsequent erythrophagocytosis; 3 to 24% of the homologous Kupffer cells had erythrophagocytosis without binding; 9 to 25% of the homologous Kupffer cells had binding alone and 16 to 66% of same had neither binding nor erythrophagocytosis (Table 4).

5.3.3.iii) Interaction between neuraminidase desialylated erythrocytes with Kupffer cells in the presence of lactose:-

Interaction of commercial neuraminidase-desialylated erythrocytes with homologous Kupffer cells in the presence of lactose resulted in 3 to 16% binding and erythrophagocytosis; 2 to 13% of the homologous Kupffer cells had erythrophagocytosis without binding; 5 to 19% had binding alone and 60% to 93% of same had neither binding nor erythrophagocytosis (Table 4).

5.3.4 iv) Interaction between untreated erythrocytes and Kupffer cells in the presence of lactose.

Following interaction of untreated erythrocytes with homologous Kupffer cells in the presence of lactose, 0 to 8% of the homologous Kupffer cells had binding and erythrophagocytosis; 0 to 6% of the homologous Kupffer cells had erythrophagocytosis without binding; 1 to 19% of the homologous Kupffer cells had binding alone and 73 to 98% of the Kupffer cells had neither binding nor erythrophagocytosis (Table 4).

5.4.0 DISCUSSION

In this study, commercial neuraminidase-treated bovine erythrocytes were bound to homologous Kupffer cells obtained from abbatoir *in vitro*. The erythrocytes were subsequently phagocytosed by 58 to 93% of the Kupffer cells in the absence of lactose. This agrees with Kolb and Kolb-Bachofen (1978) who reported that rat Kupffer cells bound commercial neuraminidase treated erythrocytes strongly but not untreated erythrocytes. They also suggested that cells adherence was mediated by a galactose specific receptor (lectin) on the Kupffer cell membrane. This was followed by subsequent erythrophagocytosis.

Also in this study, the binding and erythrophagocytosis were drastically reduced to 3 to 16% following addition of lactose (an inhibitor) during incubation. This was in agreement with the observation (Kolb and Kolb-Bachofen, 1978) in rats. The authors reported that, binding between the neuraminidase treated, hence desialylated erythrocytes and Kupffer cells was inhibited by preincubation of the macrophages (Kupffer cells) with D-galactose and related sugars, like lactose but not with unrelated saccharides.

The observed inhibition of binding and phagocytosis by lactose in the present study is further evidence to suggest that lectin plays a vital role in erythrophagocytosis both *in vitro* and *in vivo* in cattle and gives credence to the observations of Kolb and Kolb-Bachofen (1978). The lactose with its galactose end bound to the lectin of Kupffer cells hence preventing recognition of the desialylated erythrocytes by the Kupffer cells thereby inhibiting binding and erythrophagocytosis as observed in the present study. This observation is similar to previous report by Kuster and Schauer (1981) that, recognition of neuraminidase treated erythrocytes occurs by a galactosyl-specific lectin on the surface of the rat peritoneal macrophages. Such a recognition, subsequent binding and erythrophagocytosis were inhibited by lactose

when rat peritoneal macrophages were interacted with neuraminidase treated erythrocytes and Kupffer cells (Kolb and Kolb-Bachofen, 1978; Kuster and Schaur, 1981).

The observations in the present study, of 12 to 65% binding and erythrophagocytosis occurring when untreated erythrocytes were interacted with homologous Kupffer cells in the absence of lactose, while interaction of the untreated erythrocytes with their homologous Kupffer cells in the presence of lactose yielded the least observed binding and erythrophagocytosis (0 to 8%) may indicate that some of the cattle that were slaughtered at the abbatoir had subclinical infections by pathogenic organisms capable of producing neuraminidase such as *Clostridium perferinges*, *Vibrio cholera* and *Trypanosoma vivax*. It was reported that the early anaemia observed in *T. vivax* infected cattle may be due to the ability of the circulating trypanosomes to produce neuraminidase, which in turn cleaves off sialic acids, rendering the erythrocytes to be more prone to phagocytosis (Esievo, 1979; 1983). Thus, resulting in early sequestration of the erythrocytes by expanded mononuclear phagocytic system (reticuloendothelial system) for phagocytosis (Esievo, 1979; Esievo *et al.*, 1982; Esievo, 1983).

The phagocytosed untreated erythrocytes was probably desialylated by subclinical protozoan infections including subclinical trypanosomiasis, this may partly account for the binding and phagocytosis of the erythrocytes by the homologous Kupffer cells. However, Aminoff *et al.* (1977) reported that normal sialidase-untreated erythrocytes also react to a limited extent to form rosettes, this could explain the normal physiological process for the removal of senescent erythrocytes in mammals. Therefore, apart from subclinical infections one may summarise from the present study that some Kupffer cells were also bound to senescent erythrocytes and this was subsequently followed by erythrophagocytosis. In conclusion, abbatoir blood specimens may not

be ideal as normal samples for the study of pathogenic micro-organisms that are neuraminidase producers.

TABLE 4: Percentage Binding and Phagocytosis of apparently normal and sialidase-treated homologous erythrocytes by cattle Kupffer cells in the presence and absence of lactose.

Case No.	Treatment	%Binding and Phagocytosis	%Phagocytosis without binding	%Binding	%No. binding or phagocytosis.
1(*RBC1-5)	Desialylated RBC + Kupffer Cells + 10% Bovine Calf Serum	93	3	4	0
(*RBC1-5)	Untreated RBC + Kupffer cells + 10% Bovine Calf Serum	21	7	9	63
(*RBC1-2)	Desialylated RBC + Kupffer Cells + 5.0ml lactose + 10% Bovine Calf Serum	10	4	11	75
(*RBC1-2)	Untreated RBC+ Kupffer cells + 5.0ml lactose + 10% Bovine Calf Serum	5	2	7	86

2 (*RBC1-5) Desialylated RBC + Kupffer Cells + 10% bovine calf serum	90	2	8	0
(*RBC1-3) Untreated RBC + Kupffer cells + 10% Bovine calf serum	40	3	18	39
(*RBC0-1) Desialyated RBC +Kupffer cells + 5.0ml lactosc + 10% Bovine Calf Serum.	3	2	5	90
(*RBCO-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	0	1	1	98

3. (*RBC1-5) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	79	0	19	2
(*RBC1-3) Untreated RBC + Kupffer cells + 10% Bovine calf serum	36	8	24	32
(*RBC1-2) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	16	5	19	60
(*RBC1-2) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% bovive calf serum	8	0	11	81

4 (*RBC1-5) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	72	5	23	0
(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	65	10	9	16
(*RBC1-2) Desialylated RBC + Kupffer Cells + 5.0ml lactose + 10% Bovine calf serum	6	8	12	74
(*RBC0-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	2	6	19	73

5 (*RBC1-4) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	58	31	9	2
(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	34	14	25	27
(*RBC1-2) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	3	2	7	88
(*RBC1-2) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	3	6	18	73

6 (*RBC1-6) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	85	6	9	0
(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	21	24	11	44
(*RBC1-2) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	10	8	18	64
(*RBC1-2) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	6	3	10	81

7 (*RBC1-3) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	88	2	10	0
(*RBC1-2) Untreated RBC + Kupffer cells + 10 Bovine calf serum	32	5	21	42
(*RBC1-2) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	12	4	17	67
(*RBC0-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	2	2	3	93

8 (*RBC1-4) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	77	15	8	0
(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	12	10	12	66
(*RBC0-1) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	9	7	15	69
(*RBC0-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	4	4	7	85

9	(*RBC1-3) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	62	12	22	4
	(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	31	21	23	25
	(*RBC0-1) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	6	2	19	73
	(*RBC0-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	2	1	9	88

10	(*RBC1-3) Desialylated RBC + Kupffer cells + 10% Bovine calf serum	85	9	6	0
	(*RBC1-2) Untreated RBC + Kupffer cells + 10% Bovine calf serum	16	19	24	41
	(*RBC0-1) Desialylated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	4	13	9	74
	(*RBC0-1) Untreated RBC + Kupffer cells + 5.0ml lactose + 10% Bovine calf serum	0	4	8	88

* Range of number of phagocytosed RBC/Kupffer cell.

6.1. RECOMMENDATIONS:

1. It is recommended that the level of tolerance to lactose in our livestock be determined prior to its concurrent infusion or use as a base to trypanocides in the course of treatment against trypanosomiasis.

2. The use of lactose alone does not kill the parasites, therefore, it is recommended that its concurrent use with trypanocides be adopted for clearance of the parasites and prevention of destruction of the normal morphology of the various tissues and organs of the body of the animal(s) suffering from trypanosomiasis.

3. The above, may be applicable to humans suffering from trypanosomiasis(sleeping sickness and Charga's disease).

REFERENCES

1. Adam, K. M. G., Paul, J. and Zaman, V. (1971): Medical and Veterinary protozoology. E. and S. Livingstone, T. Place, Edingburge. pp. 60-80.

2. Adenowo, T. K. O. (1989): Effects of experimental *Trypanosoma vivax* infection on the estrous cycle of Maturu Yankasa ewes. M.Sc. Thesis, Ahmadu Bello University, Zaria, Nigeria. pp. 156.

3. Agu, W. E., Ige, K., Olatunde, D. S. (1986): Evaluation of Semen quality of rams infected with *Trypanosoma vivax*. Animal Reproduction Science, 11: 123-127.

4. Akpavie, S. O., Ikede, B. O., Egbunike, G. N. (1987): Ejaculate characteristics of sheep infected with *Trypanosoma brucei* and *T. vivax*: changes caused by treatment with diminazene aceturate. Research in Veterinary Science, 42: 1 - 6.

5. Aliu, Y. O. and Chineme, C. N. (1980: Isometamidium-dextran complex: toxicity and activity against *T. vivax* and *T. congolense* in rats and mice. Toxicology and Applied Pharmacology, 53: 196 - 203.

6. Allt, G., Evans, E. M. E., Evans, D. H. L. and Targett, G. A. T. (1971): Effect on infection with trypanosomes on the development of experimental allergic neuritis in rabbits. Nature (London), 233: 197-199.

7. Aminof, D., Vorder-Bruegge, W. F., Bell, W. C., Sarpolis, K. and Williams, R. (1977): Role of Sialic acid in Survival of erythrocytes in the circulation: Interaction of neuraminidase treated and untreated erythrocytes with spleen and liver at the cellular level. Biochemistry, 74: 1521 - 1524.

8. Anene, B. M. Chime, A. B., Anika, S. M. (1991): The production performance of imparted Friesian cattle under heavy trypanosoma challenge in a rain forest zone of Nigeria. British Veterinary Journal, 147: 275 - 282.

9. Anosa, V. O. and Ikede, B. O. (1976): Serum proteins, blood and plasma volumes in experimental *Trypanosoma vivax* infections of sheep and goats. Tropical Animal Health and production, 8: 14 - 19.

10. Anosa, V. O. and Isoun, T. T. (1980): Further observations on the testicular pathology in *Trypanosoma vivax* infection of sheep and goats. Research in Veterinary Science, 28: 151 - 160.

11. Anosa, V. O. and Kaneko, J. J. (1983a): Pathogenesis of *Trypanosoma brucei* infection in deer mice *(Peromyscus maniculatus)*. V. macropahage ultrastructure and function. Veterinary Pathology, 20: 617 - 631.

12. Anosa, V. O. and Kaneko, J. J. (1983b): Pathogenesis of *T. brucei* infection in deer mice (*Peromyscus maniculatus*): light and electron microscopic studies on erythrocyte pathological changes and phagocytosis. American Journal of Veterinary Research, 44: 645 - 651.

13. Anosa, V. O. and Kaneko, J. J. (1984): Pathogenesis of *Trypanosoma brucei* infection in Deer Mice *(Peromyscus maniculatus)*. Light and Electron Microscopic Study of Testicular Lesions. Veterinary Pathology, 21: 238 - 246.

14. Apted, F. J. C. (1970): Clinical manifestations and diagnosis of sleeping sickness. In: African Trypanosomiases. Ed. H. W. Mulligan, G. Allan and Unwin Ltd., London, pp. 661-683.

15. Araujo-Jorge, T. C. de and Souza, W. de (1984): Effect of carbohydrates, periodate and enzymes in the process of endocytosis of *Trypanosoma cruzi* by macrophages. Acta Tropica, 41: 17 - 28.

16. Askonas, B. A., Corsini, A. C., Clayton, C. E., Ogilrie, B. E. and Ogilrie, B. M. (1979): Functional depletion of T. and B-memory cells and other lymphoid cell subpopulations during trypanosomiasis. Immunology, 36: 313.

17. Askonas, B. A., Grosskinsky, C. M. and Sacks, D. I. (1980a): Macrophages as mediators of immunosuppression in African trypanosomiasis. In: Phagocytosis past and future (Based on conference; A centuary since Metchnikoff phagocytosis past and future) Italy Sept. 22-27.

18. Askonas, B. A., Sacks, D. L. and Grosskinsky, C. M. (1980b): Havoc in the immune system caused by African trypanosomes: studies on the active parasite product and its target cell. In: Cancer immunology and parasiteimmunology. Correlation or juxtaposition. (Colloguium, July, 28-31 Seillac, France-INSERM) Paris, France INSERM (1981) Vol. 97:341-347. Ed. Israel, L., Langrange, P. H. and Salomon, J. C.

19. Askonas, B. A. (1985): Macrophages as mediators of immunosuppression in murine African trypanosomiasis. In: The biology of trypanosomes: Current topics in microbiology and immunology, Vol. 117. pp 119-127. Berlin, Germany, Springer-Verlag.

20. Assoku, R. K. G. (1975): Immunological studies of the mechanisms of anaemia in experimental *Trypanosoma evansi* infection in rats. Internation Journal for parasitology, 5: 197-145.

21. Assoku, R. K. G., Tizard, I. R. and Nielson, K. H. (1977): Free fatty acids, complement activation and polyclonal B-cell stimulation as factors in the immunopathogenesis of African Trypanosomiasis. Lancet, ii: 956.

22. Baltz, T., Baltz, D., Grimond, C. and Pautrizel, R. (1981): Immune depression and macroglobulinaemia in experimental subchronic trypanosomiasis. Infection and immunity, 32: 179.

23. Banks, K. K. (1979): *In vitro* binding of *Trypanosoma congolense* to erythrocytes. Journal of Protozoology, 26: 103-108.

24. Banks, K. L. (1980): Injury induced by *Trypanosoma congolense* adhesion to cell membranes. Journal of parasitology, 66: 34-37.

25. Blaser, E., Jibbo, J. M. C. and McIntyre, W. I. M. (1979): A field trial of the protective effect of "Samorin" and "Berenil" in Zebu cattle under ranching conditions in Tanzania. In: International Scientific Council for trypanosomiasis Research and Control. 15th meeting, Banjul, The Gambia 1977. OAU/STRC NO.110: 383 - 386.

26. Boreham, P. e. L. (1968): Ph.D. Thesis. University of Londong.

27. Bourn, D. and Scott, M. (1978): The successful use of work oxen in agricultural development of tsetse fly infested land in Ethiopia. Tropical Animal Health and Production, 10: 191-203.

28. Boycott, A. E. and Prince-Jones, C. (1913): Experimental trypanosome anaemia. Journal of Pathology and Bacteriology, 17: 347 - 366.

29. Boyt, W. T. (1971): Trypanosomiasis control in Rhodesia. Bulletin of International Epizootiology, 76: 301-306.

30. Buscher, H. P., Casal - Stenzel, J. and Schauer, R. (1974): New sialic acids, indentification of N-glycolyl-o-acytylnueraminic acids and O-acyl groups by gas lipid chromatography. European Journal of Biochemistry, 50: 71-82.

31. Chandler, R. I. (1952): Comparative tolerance of West African N'dama cattle to trypanosomiasis. Annals of Tropical Medicine and Parasitology, 46: 127-134.

32. Choquel, P. G. (1969): Interet et utilization des bovins trypanotolerants, Thesis, Ecole Nationale Veterinaire, Alfort, Paris.

33. Charkson, M. J. (1968): Blood and plasma volume in sheep infected with *Trypanosoma vivax*. Journal of Comparative Pathology, 78: 189 - 193.

34. Clarkson, M. J. (1976): Immunoglobulin M. in trypanosomiasis. In: Pathophysiology of parasitic infection. Edited by E. J. L. Soulsby, Academic Press, New York, San Francisco: London pp. 171 - 182.

35. Clarkson, M. L., Penhale, W. J., Edwards, G. and Farrel, P. F. (1975): Serum protein changes in cattle infected with *Trypanosoma vivax*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 69: 2.

36. Clinton, B. A., Ortiz-Ortiz, L., Garcia, W., Martinez, T. and Capin, R. (1975): *Trypanosoma cruzi*: early immune responses in infected mice. Experimental Parasitology, 37: 415 - 417.

37. Coid, C. R. (1974): Comparative aspects of infection during pregnancy in intrauterine infections CIBA Foundation symposium 10 (new) series) Association of Scientific Publishers, Amsterdam, pp 117 - 129.

38. Connor, R. J. (1989): Final report of the Regional trypanosomiasis expert. Regional tsetse and trypanosomiasis control program, Malawi, Mozambique, Zambia and Zimbabwe, FGU-Kronberg consulting and Engineering GmbH. Ko.

39. Connor, R. J. (1991): The diagnosis, treatment and prevention of animal trypanosomiasis under field conditions. Programme for the control of African animal trypanosomiasis and related development: Ecological and technical aspects. proceedings of the FAO panel experts Harare, Zimbabwe, 24-26 June. pp 1-38.

40. Crossby, W. H. (1966): Red cell destruction: role of the spleen. The 11th Congress of the International Society of Haematology pp.230.

41. Danon, D., and Marikowsky, Y. (1966): Biophysical aspects of red blood cells ageing. The 11th Congress of the International Society of Haematology, pp. 394.

42. Dargie, J. D. (1978): Effects of *T. congolense* and *T. brucei* on the circulatory volumes of cattle. In: Pathogenicity of trypanosomes. Proceedings of a workshop held at Nairobi, Kenya, November, 20-23. pp. 140 - 144 edited by Losos, G. and Chouinard, A. J.

43. Dargie, J. D., Murray, P. K., Murray, M. and McIntyre, W. I.M. (1979a): The blood volume and erythrokinetics of N'dama and Zebu cattle experimentally infected with *T. brucei*. Research in Veterinary Science, 26: 245 - 247.

44. Dargie, J. D. Murray, P. K., Murray, M., Grimshaw, W. R. T. and McIntyre, W. I.M. (1979b): Bovine trypanosomiasis: the red cell kinetics of N'dama and Zebu cattle infected with *Trypanosoma congolense*. Parasitology, 78: 271 - 286.

45. De Gee, A. L. W. Shah, S. D. and Doyle, J. J. (1979): *Trypanosoma vivax*: Sequence of antigenic variations in mice and goats. Experimental parasitology, 48: 352-358.

46. Desowitz, R. S. (1959): Studies on immunity and host-parasite relationships. I. The immunological response to resistant and susceptible breeds of cattle to trypanosome challenge. Annals of Tropical Medicine and Parasitology, 53: 293-313.

47. Djabakou, K., Grundler, G., Finman, H. O. and Adomefa, K. (1985): Abortions caused by *T. congolense* (Avetonou) in N'dama and Baoule cattle. Ttryp et Prod. Anim., 4: 5 - 8.

48. Dowler, M. E., Schillinger, D. and Connor, R. J. (1989): Notes on the routine intravenous use of isometamedium in the control of bovine

trypanosomiasis on the Kenya coast. Tropical Animal Health and production, 21: 4-10.

49. Durocher, J. R., Payne, R. C. and Conrad, M. E. (1975): Role of sialic acid in erythrocyte survival. Blood, 45: 11 - 20.

50. Edwards, E. E., Judd, J. M. and Squre, F. A. (1956): Observation on trypanosomiasis in domestic animals in West. Africa. Annal of Tropical Medicine and Parasitology, 50: 223 - 241.

51. Edwards, E. E., Judd, J. M. and Squire, F. A. (1957): Observations on trypanosomiasis in domestic animals in West Africa. III. The haematological changes produced in horses by infections of *T. vivax*, *T. congolense* and *T. brucei*. Annal of Tropical Medicine and Parasitology, 51: 63 - 79.

52. Eisen, H. N. (1974): Immunology: an introduction to molecular and cellular principles of the immune responses. Harper and Row, Hagerstown Maryland, New York, Evaston, San Francisco, London, pp. 606 - 607.

53. Esievo, K. A. N. (1979): *In vitro* production of neuraminidase (sialidase) by *T. vivax*. proceedings of the 16th meeting of the OAU/STRC International council for trypanosomiasis research and control. Yaounde, Cameroon, October 29 - November 3, pp. 205 - 210.

54. Esievo, K. A. N. (1983): *Trypanosoma vivax*, stock V953: inhibitory effect of Type A. influenza virus anti-HAV8 serum on *in vitro* neuraminidase (sialidase) activity. Journal of parasitology, 69: 491 - 495.

55. Esievo, K. A. N., Jaye, A., Andrews, J. N., Ukoha, A. I., Alafiatayo, R. A., Eduvie, L. O., Saror, D. I. and Njoku, C. O. (1990): Electrophoresis of bovine erythrocyte sialic acids: existence of additional band in trypanotolerant N'dama cattle. Journal of Comparative Pathology, 102: 357 - 361.

56. Esievo, K. A. N., Moore, W. E. and Muller, R. (1982): Scanning electron microscopy of erythrocytes from *Trypanosoma vivax* infected cattle. Departments of laboratoryMedicine and Pathology, Kansas State Universtity, Manhattan, U. S. A. (Unpublished).

57. Esievo, K. A. N., and Saror, D. I. (1983): Leukocyte responses in experimental *T. vivax* infection in cattle. Journal of Comparative Pathology, 93: 165 - 169.

58. Esievo, K. A. N., Saror, D. I., Ilemobade, A. A. and Hallaway, M. H. (1982): Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *T. vivax* infection in cattle. Research in Veterinary Science, 32: 1 - 5.

59. Esievo, K. A. N., Sarror, D. I., Kolo, M. N. and Eduvie, L. O. (1986): Erythrocyte surface sialic acid concentrations in N'dama and Zebu cattle. Journal of Comparative Pathology, 96: 95 - 100.

60. Esievo, K. A. N. and Saror, D. I. (1991): Immunochemistry and immunopathology of Animal Trypanosomiasis. Protozoological abstracts, CAB international, 15: 337 - 348.

61. Eylar, E. H., Madof, M. A., Brody, O. W. and Oncley, J. L. (1962): The contribution of sialic acid to the surface charge of the erythrocyte. Journal of Biological Chemistry, 237: 1992.

62. Fiennes, R. N. T. W., Jones, E. R. and Laws, S. G. (1946): The course and pathology of *T. congolense* (Broaden) disease of cattle. Journal of Comparative Pathology, 56: 1-27.

63. Fiennes, R. N. T. W. (1954): Haematological studies in trypanosomiasis of cattle. Veterinary Record, 66: 423 - 434.

64. Fiennes, R. N. T. W. (1970): Pathogenesis and Pathology of Animal trypanosomiasis. In: The African trypanosomiases. Edited by H. W.

Mulligan. George Allen and Unwin Ltd., 40 Museum Street, London. pp 729 - 750.

65. Fifrer, J. and Askonas, B. A. (1982): *Trypanosoma brucei* infection stimulates receptor-mediated phagocytosis by murine peritoneal macrophages. Infection and Immunity, 37: 1281 - 1284.

66. Fio-Ngaindiro, G. (1987): Development of Animal industry and improvement of Animal in the Central African Republic. Rev. Sci et Techn., 6: 955 - 967.

67. Fluck, D. J. and Hopkins, J. S. (1988): Chemotheropy and chemoprophylaxis of bovine trypanosomiasis. In: International Scientific Council for Trypanosomiasis Research and Control. Proceedings of the 19th meeting, Lome, 1987. Publication No. 114 OAU/STRC, Nairobi, pp 302 - 313.

68. Ford, J. (1970): Control of populations of Glossina: Introduction to control methods. in: The African Trypanosomiases. Edited by H. W. Mulligan. George Allen and Unwin Ltd., 40 Museum street, London, WCIA, ILU pp. 453 - 455.

69. Goldman R. and Cooper, R. A. (1975): Concanavalin - A mediated attachment and ingestion of red blood cells by macrophages. Experimental Cell Research, 95: 223 - 234.

70. Goodwin, L. G., Green D. G., Guy, M. W. and Voller, A. (1972): Immunosuppression during trypanosomiasis. British Journal of Experimental Pathology, 53: 40-43.

71. Greenwood, B. M., Brown, J. C., DeJesus, D. G. and Holborow, E. J. (1971): Immunosuppression in murine malaria. KK. The effects on reticuloendothelial and germinal centre function. Clinical Experimental immunology, 9: 345 - 354.

72. Greenwood, B. M. (1974a): Immunosuppression in malaria and trypanosomiases. Parasites in the immunized hosts: mechanism of survival. CIBA Foundation Symposium, 25: 137 - 146.

73. Greewood, B. M. (1974b): Possible role of B.cell mitogen in hypergammaglobulinaemia. Lancet. i: 435.

74. Greenwood, B. M. (1976): Immunosuppression in African trypanosomiasis. World health Organization, TRYP/WP/76, 16: 1-3.

75. Grosskinsky, C. M., Ezekowitz, R. A. B., Berton, G., Gordon, S. and Askonas, B. A. (1983): Macrophage activation in murine African trypanosomiasis. Infection and Immunity, 39: 1080 - 1086.

76. Grundler, G. A. and Djabakou, K. (1985): The impact of trypanosomiasis on the quality of sperm. Tryp et prod. Anim, 4: 9 - 14.

77. Grundler, G., Djabakou, K., Hanichen, T., and Ademofa, K. (1988): Testicular lesion in bulls infected with *T. congolense* Tryp et Prod. Anim, 4: 17 - 24.

78. Guleed, H. A. and Bornstein, S. (1987): Pilot study of the health of Somali camel herds. Camel Forum, Workshop paper, 23: 1-10.

79. Hendy, C. R. C. (1988): The effects of trypanosomiasis prophylaxis and antihelmintic treatment in goats under traditional management in southern Tanzania. In: Livestock Production in Tsetse affected areas of Africa. proceedings of a meeting held 23 - 27 November, 1987, Nairobi, Kenya. ILCA/ILRAD. Nairobi, pp. 289 - 309.

80. Herbert, W. J., Inglis, M. D. (1973): Immunization of mice against *T. brucei* infection by the administration of released antigen adsorbed to erythrocytes. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67: 268.

81. Holmes, P. B. and Jennings, F. W. (1976): The effct of treatment on the anaemia of African trypanosomiasis. In: Pathophysiology of Parasitic Infection. Academic Press, New York, San Francisco, London, pp. 199 - 210. Ed. by Soulsby, E. J. L.

82. Huan, C. N., Webb, L., Lambert, P. H. and Miescher, P. A. (1975): Pathogenesis of the anaemia in African trypanosomiasis: Characterisation and purification of haemolytic factor. Schweiz Med Wschr, 105: 1582 - 1583.

83. Hudson, K. M., Byner, C., Freeman, J. and Terry T. R. (1976): Immunosuppression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature, 264 : 256.

84. Ibrahim, N. D. G., Umar, I., Omage, J.J. Shugaba, A., Kadima, K. B., Igbokwe, I. O. Agbede, R. I. S., Njoku, C. O., Saror, D. I. and Esievo, K. A. N. (1994): Erythrophagocytosis of acute ruminant trypanosomiasis *Trypanosoma vivax* is lectin based. Journal of Comparative Pathology, submitted.

85. Ige, K. and Amadu, A. A. (1977): Studies on the Pathogenesis of trypanosomiasis for N'dama cattle. In: ISCTRC 14th meeting, Dakar, Senegal 1975. pp 155 - 156. OAU/STRC No. 109.

86. Ikede, B. O. and Losos, g. J. (1972): Congenital transmission of *Trypanosoma vivax* in sheep. British Veterinary Journal, 128: i - ii.

87. Ikede, B. O., Lule, M. and Terry, R. J. (1977): Anaemia in trypanosomiasis: mechanisms of erythrocyte destruction in mice infected with *T. congolense* and *T. brucei*. Acta Tropica, 34: 53 - 60.

88. Ikede, B. O. (1979): Genital lesions in experimental chronic *T. brucei* infection in rams. Research in Veterinary Science, 26: 145 - 151.

89. Ikede, B. O. and Akpavie, S. O. (1982): Delay in resolution of trypanosome induced genital lesions in male rabbits infected with *T. brucei* and treated with diminazene aceturate. Research in Veterinary Science, 32: 374 - 376.

90. Ikede, B. O., Elhassan, E. and Akpavie, S. O. (1988): Reproductive disorders in African trypanosomiasis: a review. Acta Tropica, 45: 5 - 10.

91. Ilemobade, A. A. and Balogun, T. G. (1981): Pig trypanosomiasis: effects of infection on feed intake, liveweight gain and carcass traits. Tropical Animal Health and Production, 13: 128 - 136.

92 Ing, T. S. G. A. M., Van den, Zwart, D., Miert, A. S. J. R. A. M. van, and Schotman, A. J. H. (1976): Clinicopathological and pathomorphological observaions in *T. vivax* infection in cattle. Veterinary Parasitology, 2: 237 - 250.

93. Igram, D. G. and Soltys, M. A. (1960): Immunity in trypanosomiasis: IV. Immunoconglutinin in animals infected with *T. brucei*. Parasitology, 50: 231 - 239.

94. Isoun, T. T. and Esuruoso, G. O. (1972): Pathology of natural infection of *T. vivax* in cattle. Nigerian Veterinary Journal, 2: 42-45.

95. Isoun, T. T. and Anosa, V. O. (1974a): Lesion in the reproductive organs of sheep and goats experimentally infected with *T. vivax*. Trop und Parasit 25: 469 - 476.

96. Isoun, T. T. and Anosa, V. O. (1974b): The effect of experimental infection of *T. vivax* on the reproductive organs of sheep and goats - a preliminary reports. Nigerian Journal of Animal Production, 1: 143 - 150.

97. Itty, P., Chema, S., d'leteren, G. D. M., Durkin J., Leak, S. G. A., Machl, J. H. H., Maloo, S. H., Mukendi, F., Nagda, S. M., Raieya, J. M., Thrope, W. and Trail, J. C.M. (1988): Economic aspects of cattle production and of chemoprophylaxis for control of trypanosomosis in village East African Zebu cattle in Kenya. In: Livestock production in Tsetse Affected Areas of Africa. Proceedings of a meeting held in Nairobi, 1987. ILCA/ILRAD, Nairobi, 360 - 376.

98. James, D. M. (1978): Prophlactic activity in rodents of trypanocides complex with dextran. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72: 472 - 476.

99. Jancik, J. M., Schauer, R., Andres, K. H. and Von During, M. (1978): Sequestration of neuraminidase treated erythrocytes: Studies on its topographic morphologogical and immunological aspects. Cell and Tissue Research, 186: 209 - 226.

100. Jayawardena, A. N. and Waksman, B.H. (1977): Suppressor cells in experimental trypanosomiasis. Nature, 265: 539.

101. Jennings, F. W., Murray, P.K., Murray, M. and Urquhart, G. M. (1974). Anaemia in trypanosomiasis: Studies in rats and mice infected with *T. brucei*. Research in Veterinary Science, 16: 70-76.

102. kantzler, G. B., Lauteria, S. F., Cusimano, C. L. Lee, J. D., Ganguly, R. and Waldman, R. H. (1974): Immunosuppression during influenza virus infection. Infection and Immunity, 10: 996 - 1002.

103. Kanyari, P. W. N., A,lonby, E. W., Wilson, A. J. and Munyua, W. K. (1983): Some economic effects of trypanosomiasis in goats. Tropical Animal Health and production, 15: 153 - 160.

104. Kobayashi, A. and Tizard, I. R. (1976): The response to *T. congolense* infection in calves: determination of immunoglobulins IgG1, IgG2,, IgM and C3 levels and the complement fixing antibody titres during

the course of infection. Tropenmedizin Und Parasitologie, 27: 411-417.

105. Kobayashi, A., Tizard, I. R. and Woo, P. T. K. (1976): Studies on the anaemia in experimental African trypanosomiasis: 11. The pathogenesis of the anaemia in calves infected with *T. congolense*. American Journal of Tropical Medicine and Hygiene, 25: 401-406.

106. Kolb, H. and Kolb-Bachofen, V. (1978): A lectin - like receptor on mammalian macrophages. Bio-medical Biophysical Research Communications, 85: 678 - 683.

107. Kuster, J. M. and Schauer, R. (1981): Phagocytosis of sialidase - treated rat erythrocytes: evidence of a two-step mechanism. Hoppe-Seyler's Zeitschrift fur physiologisch Chemie, 362: 1507 - 1514.

108. Lansteiner, K. and Raubitschek, H. (1907): Beobachtungen Uber Hamolyse and Hamagglutination. Centralbl. F. Bakt, 45: 660 - 667.

109. Laveran, A. and Mesnil, F. (1907): Trypanosomes and trypanosomiasis: Baillere Tindal. London. pp146.

110. Liu, A. Y. C., Ploeg, L. H. T., Van der Rijsewijk, F. A. M. and Borst, P. (1983): The transposition unit of variant surface glycoprotein gene 118 of *T. brucei*. Presence of repeated elements at it's border and absence of promoter-associated sequences. Journal of Molecular Biology, 167: 57 - 75.

111. Llewely, C. A., Luckins, A. G., Munro, C. D., and Perrie, J. (1987): The effect of *T. congolense* infection on the oestrous cycle of the goat. Britishm Veterinary Journal, 143: 423 - 431.

112. Llewelyn, C. A., Munro, C. D. Luckins, A. G., Jordt, T., Murrary, M. and Lorenzini, E. (1988): The effects of *T. congolense* infection on the oestrous cycle of the Boran cow. British Veterinary Journal, 144: 379 -387.

113. Logan, L. L., Goodwin, J. T., Tembely, S. and Craig, T. M. (1984): Maintaining Zebu, Muturu cattle in tsetse infested area of Mali. Tropical Animal Health and production, 16: 1 - 12.

114. Losos, G. J. and Ikede, B. O. (1972): Review of pathology of the diseases in domestic and laboratory animals caused by *T. congolense, T. brucei, T. rhodesiense and T. gambiense*. Veterinary Pathology, *9*: 1-71.

115. Lor, K. F., Pholpark, S., Siriwan, P., Leesirikul, N., Srikitjakara, L. and Staak, C. (1986): *Trypanosoma evansi* infection in buffaloes in North-East Thailand. 11 Abortions. Tropical Animal Health and Production, 18: 103-108.

116. Luckins, A. G., Llewelyn, C., Munro, C.D. and Murray, M. (1986): Effects of pathogenic trypanosomes on the mammalian reproductive system. Nuclear and related techniquest in animal prouction and health. proceedings of an international Atomic Energy Agency and the Food and Agricultural Organisation, Vienna, Austria, pp. 351-353.

117. Mackenzie, P. K. I. and Cruickshank, J. G. (1973): Phagocytosis of erythrocytes and leukocytes in sheep infected with *T. congolense*. Research in Veterinary Science, 15: 256 - 262.

118. Mackenzie, P. K. I., Boyt, W. R.., Nesham, V. W. and Purue, E. (1978): The etiology and significance of the phagocytosis of erythrocytes and leucocytes in sheep infected with *T. congolense* (Broden, 1904). Research in Veterinary in Veterinary Science, 24: 4-7.

119. Maloo, S. H., Chema, S., Connor, R., Durkin, J., Kimotho, P., Maehl, J. H. H., Mukendi, F. Murray, M., Rarieya, J. M. Mukendi, F., Murray, M., Rarieya, J. M. and Trail, J. C. M. (1988): The use of Chemoprophylaxis in East African Zebu village cattle exposed to trypanosomiasis in muhaka, Kenya. In: Livestock Production in

Tsetse affected areas of Africa. Proceedings of a meeting of the African Trypanotolerant Livestock Network, held in Nairobi, Kenya 23-27 November, (1987). English Press, Nairobi, Kenya. pp. 283 - 288.

120. Mamo, E. and Holmes P. (1975): The erythrokinetics of Zebu cattle chronically infected with *T. congolense*. Research in Veterinary Science, 18: 105 - 106.

121. Mamo, E., Holmes, P. and Lemma, A. (1977): Some aspects of the anaemia in bovine trypanosomiasis. 11. Erythrocyte survival studies in cattle infected with *T. congolense* using 51_{Cr}-labelled red blood cells. Ethiopian Veterinary Bulletin, 3: 15-25.

122. Mansfield, J. M. (1978): Commentary: Immunobiology of African trypanosomiasis. Cellular Immunology, 39: 204.

123. Mansfield, J. M. and Wallance, J. H. (1974): Suppression of cell mediated immunity in experimental African trypanosomiasis. Infection and Immunity, 10: 335.

124. Marikowsky, Y., Danon, B. and Katchalsky, A. (1966): Agglutination by polylysin of young and old red blood cells. Biophisica Biochemica Acta, 124: 154.

125. Mason, I. L. (1951): The classification of West African Livestock. Farnham Royal. Commonwealth Agricultural Bureaur.

126. Maxie, M. G., Losos, G. J. and Tabel, H. (1976): A comparative study of the haematological aspects of the diseases caused by *Trapanosoma vivax* and *Trypanosoma congolense* in cattle. In: Pathophysiology of parasitic infection. Academic Press. New York, San-Francisco, London, pp. 183-198. Ed. by Soulsby, E. J. L.

127. Maxie, M. G., Losos, G. J. and Table, H. (1979): Experimental bovine trypanosomiasis (*Trypanosoma vivax* and *Trypanosoma congolense*) 1.

Symptomatology and clinical pathology. Tropenmedizin and Parasitologie, 30: 274-282.

128. Maxie, M. G. and Valli, V. E. O. (1978): Pancytopenia in bovine trypanosomiasis. In: Pathogenicity of trypanosomes. Proceedings of workshop held in Nairobi, Kenya, November, 20-23, pp. 135-136. Ed. by Losos, G. and Chouinard, A. J.

129. Michels, P. A. M., Liu, A. Y. C., Bernards, A., Sloof, P., Bijl, M. M. W. van der, Schinkel, A. H., Menke, H. H., Borst, P., Veeneman, G. H., Trop, M. C. and Boom, J. H. van (1983): Activation for the genes for variant surface glycoproteins 117 and 118 in *Trypanosoma brucei*. Journal of Molecular Biology, 166: 537-556.

130. Mohammed, G.(1991): Comparative pathogenicity of trypanosomes of the subgenus Nannomonas with special reference to a new specie. Ph. D. Thesis, University of Bristol. U. K.

131. Mohr, M., Kolb, H., Kolb-Bachofen, V. and Schlepper-Schafer, J. (1987): Recognition of Xenogeneic erythrocyte; the CalNAc/Cal-particle receptor of rat liver mediates or participates in recognition. Biology of the cells 60. 217-224.

132. Moulton, J. E. and Sollod, A. E. (1976): Clinical, serologic and pathologic changes in calves with experimentally induced *Trypanosoma brucei* infection. American Journal of Veterinary Research, 37: 791 - 802.

133. Muller, E., Franco, M. W. and Schauer, R. (1981): Involvement of membrane galactose in the *in vivo* and *in vitro* sequestration of desialylated erythrocytes. Hoppe-Seyler's Zeitschrift Fur Physiologisch Chemie, 362: 1615 - 1620.

134. Muller, E., Schroder, C., Franco, M. W., Shukla, A. K. and Schauer, R. (1983a): Involvement of a B-galactose-specific macrophage lectin in binding and phagocytosis of erythrocytes. Lectin 111. Walter de

Gruyter and Co., Berlin, New York. pp 435-442 (edited by Bog-Hansen T. C., Spengler, G. A.).

135. Muller, E., Schroder, C., Schauer, R. and Sharon, N. (1983b): Binding and Phagocytosis of sialidase - treated rat erythrocytes by a mechanism independent of Opsonins. Hoppe-Seyler's Zeitschrift fur physiologisch Chemie, 364: 1419 - 1429.

136. Muller, E. and Schauer, R. (1983): Factors influencing binding and phagocytosis of sialidase-treated erythrocytes by bomologous rat peritoneal macrophages. Glycoconjugates. In: Proceedings of the 7th Symposium on Glycoconjugates Lund-Ronneby, July 17-23 pp. 667-668. Ed. by Chester, M. A., Heingard, D., Lundbland, A. and Svensson, S. J.

137. Murray, M., Murray, P. K., Jennings, F. W., Fihser, E. W., Urquhart, G. M. (1974): The Pathology of *T. brucei* infection in rat. Research in Veterinary Science, 16: 77-84.

138. Murray, P. K., Jennings, F. W., Murray, M. and Urquhart, G. M. (1974a): The nature of immunosuppression in *T. brucei* infections in mice. 1. The role of the macrophage. Immunology, 27: 815.

139. Murray, P. K., Jennings, F. W., Murray, M. and Urquhart, G. M. (1974b): The nature of immunosuppression in *T. brucei* infection in mice. 11. The role of T and B lymphocytes. Immunology, 27: 825.

140. Murray, P. K., Murray, M., Wallace, M., Morrison, W. I. and McIntyre (1977): Trypanosomiasis in N'dama and Zebu cattle. Influence of weight of infction on the severity of the disease. Proceedings of the 15th meeting of the OAU/STRC. International Scientific Council for Trypanosomiasis Research and Control, Banjul, The Gambia, pp. 482-487.

141. Musoke, A. J., Nantulya, V. M., Brbet, E. F., Kironde, F., McGuire, T. C. (1981): Bovine immune response to African Trypanosomes:

specific antibodies to variable surface glycoproteins of *T. brucei*. Parsite immunology, 3: 97.

142. Mutayoba, B. M., Gombe, S., Kaaya, G. P. and Waindi, E. N. (1985): The effects of chronic experimental *T. congolense* infection on the ovaries, pitluitary, thyroid, and adrenal glands in female goats. Research in Veterinary Science, 44: 140-145.

143. Mutayoba, B. M., Gombe, S., Waindi, E. N. and Kaaya, G. P. (1988): Depression of overian function and plasma progesterone and estradiol-17 in female goats chronically infected with *T. congolense*. Acta Endocrinology, 117: 477-484.

144. Nantulya, V. M., Muslke, A. J., Rurangirwa, F. R., Borbert, A. F., Ngaira, J. m. and Katende, J. M. C. (1982): Immunodepression in African Trypanosomiasis: the role of antigenic competition. Clinical and Experimental Immunology, 47: 234.

145. Nantulya, V. M. Muslke, A. J., Rurangirwa, F. R., Saigar, N. and Minja, S. H. (1987): Monoclonal antibodies that distinguish *T. congolense*, and *T. brucei*. Parasite Immunology, 9: 421-431.

146. Naylor, D. C. (1971): The haematology and histopathology of *T. congolense infection* in cattle. Tropical Animal Health and production, 3: 159-168.

147. Nguyen Haun, C., Webb, L., Lambert, P.H. and Miescher, P. A. (1975): Pathogenesis of the anaemia in African trypanosomiasis: characterization and purification of haemolytic factor. Schweizerishe Medizinische Wochenschrift, 105: 1582-1583.

148. Nielsen, K., Scheppard, J., Holmes, W. and Tizard, I. (1978a): Experimental bovine trypanosomiasis: changes in serum immunoglobulins, complement and complement components in infected animals. Immunology, 35: 817-826.

149. Nielsen, K., Sheppard, J. and Holes, W. (1978b): Increased susceptibiligy of *T. lewisi* infected decomplemented rats to *Salmonella typhimurium*. Experientia, 34: 118-119.

150. Nielson, K., Sheppard, J., TiZard, I. and Holmes, W. (1978c): Dirct activation of complement by trypanosomes. Journal of Parasitology, 4: 544-546.

151. Nielsen, K., Tizard, I. R. and Sheppard, J. (1978d): Complement in experimental trypanosomiasis: Pathogenicity of trypanosomes. proceedings of a workshop held in Nairobi, Kenya, November 20-23, pp. 94-102.

152. Njoku, A. R., Dolan, R. B., Sayer, P. D., Wilson, A. J. and Alushula, H. (1985): Strategic chemoprophylaxis for the control of bovine trypanosomiasis. International Scientific Council for trypanosomiasis Research and Control. Proceedings of the 18th meeting, Harare, Publication No.113 OAU/STRC, Nairabi. 199-204.

153. Njoku, C. O. (1985): Why animal health and disease investigation in food animal production in Nigeria? Paper, 10th Annual Conference of the Nigerian Society for Animal Production.

154. Nohle, U. I., Boau, J. M. and Schauer, R.(1982): European Journal of Biochemistry, 126: 543 - 548.

155. Ogwu, D., Njoku, C. O., Osori, D. I. K., Ezeokoli, C. D. and Kumi-Diaka, J. (1984): Effects of experimental *T. vivax* infection in fertility of heifers. Theriogenology, 22: 625-633.

156. Ogwu, D., Njoku, C. O. and Osori, D. K. (1986): Effects of Experimental *T. vivax* infection on first, second and third trimester pregnancy in heifers. Theriogenology, 25: 383-398.

157. Ogwu, D. and Njoku, C. O. (1987): Effrects of pregnancy on clinical manifestations of bovine trypanosomiasis. Veterinary parasitology, 24: 25-33.

158. Ogwu, D. and Njoku, C. O. (1991): Genital lesions in experimental *T. congolense* infection in heifers. Animal Reproductions Science, 26: 1-11.

159. Ogunyemi, O., and Ilemobade, A. A. (1989): Prophylaxis of African Animal trypanosomiasis: A review of some factors that may influence the duration of isometamidium and diminazene. A paper presented to the 20th meeting of the International Scientific Research and Control, Mombasa, Kenya.

160. Omage, J. J., Umar, I. A., Shugaba, A. Igbokwe, I. O., Ibrahim, N. D. G., Kadima, K. B., Ameh, D. A., Agbede, R. I. S., Onyemelukwe, G. C., Saror, D. I. and Esievo, K. A. N. (1996): Involvement of erythrocyte membrane galactose in early anaemia observed in experimental bovine trypanosomiasis *T. vivax)*. Journal of Comparative Pathology (sumitted).

161. Ormeroid, W. D. (1970): Pathogenesis and Pathology of trypanosomiasis: In: The African Trypanosomiasis. Ed. by Mulligan, H. W. and Allan, G. Unwin Ltd., London. pp 287-289.

162. Ortiz-Ortiz, L. , Ortega, T., Capia, R. and Martinex, T. (1976): Enhanced mononuclear phagocytic activity during *Trypanosoma cruzi* infection in mice. International Archieves of Allergy and Applied Immunology, 50: 232-242.

163. Pereira, M. E. A.(1983): A developmentally regulated neuraminidase activity in *T. cruzi*. Science, 219: 1444 - 1446.

164. Pereira, M. E. A. and Hoff, R. (1986): Heterogeneous distribution of neuraminidase activity in strains and clones of *Trypanosoma cruzi* and

its possible association with parasite myotropism. Molecular and Biochemical Parasitology, 20: 183-189.

165. Ramos, C., Lamoyi, E., Feoli, M., Rodriquez, M., Perez, M. and Ortiz-Ortiz, L. (1978): *Trypanosoma cruzi*: Immunosuppressed response to different antigens in the infected mouse. Parasitology, 45: 190 - 199.

166. Rautenberg, P., Reinwald, E. and Risse, H. J. (1981): Sialic acids are responsible for the charge heterogeneity of the variant surface glycoprotein of *Trypanosoma congolense*. Molecular and Biochemical Parasitology, 4: 129 - 138.

167. Reed, W. P., Olds, J. W. and Kisch, A. L. (1972): Decreased skin test reactivity associated with influenza. Journal of Infectious Diseases, 125: 398.

168. Reinwald, E., Rautenberg, P. and Risse, H. J. (1979): *Trypanosoma congolense*: mechanical removal of the surface coats. Experimental Parasitology, 48: 384-397.

169. Reuter, G., Pfeil, R., Stoll, S., Schauer, R., Kamerling, J. P., Versluis, C. and Vliegenthart, J. F. G. (1983): Indentification of new sialic acids derived from glycoprotein of bovine submandibular gland. European Journal of Biochemistry, 134: 139-143.

170. Roper, N. (1978) In: Pocket Medical Dictionary. 13th Edition, Hazel, Watson and Winey Ltd., Aylsbury, Britain pp157.

171. Rottino, A. and Angers, J. (1961): Electrophoetic mobility of red blood cells during human pregnancy. American Journal of obstetrics and Gynecology, 82: 1302.

172. Rurangirwa, F. R., Musoka, A. J., Nantulya, V. M. and Tabel, H. (1983): Immune depression in bovine trypanosomiasis: effects of

acute and chronic trypanosoma vivax infections on antibody response to *Brucella abortus* vaccine, Parasite Immunology, 5: 267 - 276.

173. Sacks, D. I. and Askonas, B. A. (1980): Trypanosome-induced suppression of anti-parasite response during experimental African trypanosomiasis. European Journal of Immunology, 10: 971-974.

174. Sacks, D. I., Bancroft, G., Evans, W. H. and Askonas, B. A. (1982): Incubation of trypanosome derived mitogenic and immunosuppressive products with peritoneal macrophages allows recovery of biological activities from soluble parasitic fractions. Infection and Immunity, 36: 160-168.

175. Sacks D. I., Grosskinsky, C. M. and Askonas, B. A. (1980). Immune dysfunction caused by African trypanosomes: Effect on parasite specific rsponses and analysis of active fractions. In: The host-invader interplay. Proceedings of the 3rd International Sympsoim on the biochemistry of parasites and host - parasite relationships. Beertse, Belgium, 30 June to 3 July. Amsterdam. The Netherlands, Elsevier/North Holland Biomedical Press. pp 335 - 338. Ed. by Van den Bossche, H.

176. Saror, D. I. (1975): Heamatology, Serum Iron and Iron-Binding Capacity of Apparently Normal and Trypanosoma Infected Zebu Cattle. Ph.D. Thesis. ABU, Zaria.

177. Saror, D. I. (1980): Observations on the course and pathology of *Trypanosoma vivax* in red Sokoto goats. Research in Veterinary Science, 28: 36-38.

178. Saror, D. I. (1980): Observations on the course and pathology of *Trypanosoma vivax* in red Sokoto goats. Research in Veterinary Science, 28: 36-38.

179. Schauer, R. (1983): Sialic acids as potential determinants on differentiation antigens. Biochemical Society Transactions, Vol. 11. 602nd. Meeting. London. pp. 270-271.

180. Schauer, R., Shukka, A. K., Schroder, C. and Muller, E. (1984): Anti-recognition function of sialic acids. Studies with erythrocytes and macrophages. Pure and Applied Chemistry 56: 907-921.

181. Schauer, R., Busher, H. P. and Gasals-Stenzel, J. (1974): Sialic acids their analysis and enzymic modifications in relation to the synthesis of submandibular gland glycoproteins. Biochemical Society Symposium, 40: 87-116.

182. Schauer, R., Reuter, G., Muhipfordt, H., Andrade, A. F. B., Pereira, M. E. A. (1983): The occurance of N-Acetyl-and N-glycolyl-neuraminic acid in T. cruzi, Hoope-Seyler's Zeitschrift fur physiologisch Chemie, 364: 1053-1057.

183. Schauer, R. C. (1978): Characterization of sialic acids. Methods in Enzymology, 50: 64 - 89.

184. Seaman, G. W. F., and Unlenbruck, G. (1963): The surface structure of erythrocytes from some animal sources. Arch Biochemi Biophys, 100: 493.

185. Sekoni, V. O., Kumi-Diaka, J., Saror D. I., and Njoku, C. O. (1988): The effect of *T. vivax* and *T. congolense* infection on the reaction time and semen characteristics in the Zebu bull. British Veterinary Journal, 114: 388-394.

186. Senomi, V. O., Saror, D. I., Njoku, C. O., Kumi-Diaka, J. and Opaluwa, G. I. (1990a): Comparative haematological changes following *T. vivax* and *T. congolense* infections in Zebu bulls. Veterinary Parasitology, 35: 11-19.

187. Sekoni, V. O., Njoku, C. O., Saror, D. I., Sanusi, A., Oyejola, B. and Kumi-Diaka, J. (1990b): Effect of chemotherapy on elevated ejaculation time and deteriorated semen characteristics consequent to bovine trypanosomiasis. British Veterinary Journal 146: 368 – 373.

188. Sekoni, V. O., Njoku, C. O. Kumi-Diaka, J. and Saror, D. I. (1990c): Pathological changes in male genetalia of cattle infected with *T. vivax* and *T. congolense*. British Veterinary Journal, 146: 175–180.

189. Sekoni, V. O., Saror, D. I., Njoku, C. O. and Kumi-Diaka, J. (1990d): Elevation of morphological abnormalities of spermatozoa in the semen of Zebu bulls consequent to *T. vivax* and *T. congolense* infection. Theriogenology, 33: 925–936..

190. Sekoni, V.O., Saror, Di. I., Njoku, C. O. and Kumi-Diaka, J. (1991): Effect of novidium (Homidium chloride) chemotherapy on elevated spermatozoa morphological abnormalities in the semen of Zebu bulls infected with *T. vivax* and *T. congolense*. Animal Reproduction Science, 249 – 258.

191. Sekoni, V. O. (1992): Effect of *T. vivax* infection on semen characteristics of Yankasa Rams. British Veterinary Journal, 148: 501–506.

192. Sekoni, V. O. (1993): Elevated sperm morphological abnormalities of Yankasa rams consequent to *T. vivax* infection. Animal Reproduction Science, 31: 243–248.

193. Sekoni, V. O. (1994): Reproduction disorders caused by animal trypanosomiasis: A review: Theriogenology, 557 – 570.

194. Shugaba, A., Umar, I., Omage, J., Ibrahim, N. D. G., Andrew, J., Ukoha, A. I., Saror, D. I. and Esievo, K. A. N. (1994): Biochemical differences (O.-Acetyle and Glycolyl Groups) in erythrocytes surface sialic acids of Trypanotolerant N'dama and Trypanosusceptible Zebu cattle. Journal of Comparative Pathology,. 110: 91–95.

195. Soulsby, E. J. L. (1968): Helminths, Arthropods and protozoa of Domesticated Animals. Six Edition. the Williams and Wilkins Company, Baltimore, U. S. A. pp. 1-809.

196. Stephen, L. E. (1970): Clinical mainfestation of Trypanosomiasis in livestock and other domestic animals. In: The African Trypanosomiasis. Ed. by Mulligan, H. W. and Allan, G. Unwin Ltd., London. pp. 779 - 794.

197. Tabel, H., Ruwangirwa, F. R. and Losos, C. J. (1978): Is the anaemia in bovine trypanosomiasis caused by immunologic mechanism? In: Pathogenicity of trypanosomes. Preceedings of a workshop held at Nairobi, Kenya, November 20-23, pp. 91-93. Ed. by Lososm G. and Chouinard, A.

198. Takayanagi, T. and Enriquex, G. L. (1973): Effects of the 1gG and 1gM immunoglobulins in *T. gambiense* infections in mice. Journal of Parasitology, 59: 644-647.

199. Takayanagi, T., Nakatake, Y. and Enriquez, G. L. (1974): Attachment and ingestion of *T. gambiense* to macrophages by specific antiserum. Journal of Parasitology, 60: 336-339.

200. Takken, W., Taylor-Lewis, E. G. and Woodford, M. H. (1988): Field studies on animal trypanosomiasis in Mozambique. Effectiveness of the prophylactic drugs isometamidium chloride and pyrithidium bromide. Tropical Animal Health and production, 20: 243-225.

201. Terry, R. J., Freeman, J., Hudson, K. M. and Longstaffe, J. A. (1973): Immunoglobulin M. production and immunosuppression in trypanosomiasis: a linking hypothesis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67: 263.

202. Tizards, I. R., Holmes, W. L. and Nielsen, K. (1978): Mechanisms of the anaemia in trypanosomiasis: studies on the role of the haemlytic

fatty acids derived from *T. congolense*. Tropenmediziin und Parasitologie, 29: 108-114.

203. Tizard, I. R., Holmes, W. L., York, D. A. and Mellors, A. (1977): The generation and identification of haemolysin of *T. congolense*. Experientia, 33: 901-902.

204. Trail, J. C. M., Murray, M., Sones, K., Jibbo J. M. C., Durkin, J. and Light, D. (1985): Boran cattle maintained by chemorprophylaxis under trypanosomiasis risk. Journal of Agricultural Science, Cambridge, 105: 147 -166.

205. Urquhart, G. M., Murray, M. and Jennings, F. W. (1972): The immune response to helminth infection in trypanosome infected animals. Transactions Royal Society of Tropical Medicine and Hygiene, 66: 342-343.

206. Valli, V. E. O., and Forsberg, C. M. (1979): The pathogenesis of *T. congolense* infection in calves. V. quantitative histological changes. Veterinary pathology, 16: 334 - 368.

207. Valli, V. E. O., Forsberg, C. M. and Mcsherry, B. J. (1978): The pathogenesis of *T. congolense* infection in calves. 11. Anaemia and erythroid response. Veterinary Pathology, 15: 732-745.

208. Van Den Ingh, T. S. G. A. M., Zwart, d., Schofman, A. J. H., Van Miert, A. S. J. P. A. M. and Veenendaal, G. H. (1976): The pathology and pathogenesis of *T. vivax* infection in goats. Research in Veterinary Science, 21: 264-270.

209. Veh, R. W., Saunder, M., Haverkamp, J. and Schauer, R. (1979): Demonstration of O-acetyl groups in ganglioside-bound sialic acids and their effect on the action of bacterial and mammalian neuraminidases. Glycoocnjugates Research, 1: 557-559.

210. Vohradsky, F. (1971): Clinical signs, daily rate of infection, physical changes of the blood and pathomorphological changes in cattle artificially infected with *T. vivax*. Rev. Elev. Med. Vet. Pays, Trop., 24: 251-263.

211. Waitumbi, J. N. and Connor, R. J. (1991): Epidemiology and control of camel trypanosomiasis in Northern Kenya. In: OAU/STRC, 15: 7082, p. 301 Abstract.

212. Weitz, B. G. F. (1970): Infection and resistance. In: The African Trypanosomiases pp. 97.124.

213. Whitelaw, D. D., Bell, I. R., Holmes, P. H., Maloo, S. K. Hirumi, H., Urquhart, G. M. and Murray, M. (1986): Isometamidum chloride prophylaxis against *T. congolense* challenge and the development of immune responses in Boran cattle. Veterinary Record, 118: 722-726.

214. Wilson, A. J., Lee Roux, J. G., Paris, J., Davidson, C. R. and Gray, A. R. (1975): Observations on a herd of beef cattle, maintained in a tsetse area. I. Assessment of chemotherapy as a method for the control of trypanosomiasis. Tropical Animal Health and production, 7: 187-199.

215. Yaari, A. (1969): Mobility of human red blood cells of different age groups in an electric field. Blood, 33: 159.

APPENDIX I:

PATHOGENICITY[1] OF SALIVARIAN TRYPANOSOMES IN LIVESTOCK

Trypanosome Donkeys Subgenus	Trypanosome species	Cattle	Goats	Sheep	Pigs	Horses
Trypanozoon	*T. brucei*[2]	+	++	++	+	+++	++
	T. evansi[3]	++	+	+	++	+++	++
	T. equiperdum[4]	-	-	-	-	+++	++
Nannomonas	*T. congolense*	+++	++	++	+	++	++
	T. Simiae	-	+	+	+++	-	-
Duttonella	*T. vivax*	+++	++	++	-	++	+
Pyenomones	*T. suis*[5]	-	-	-	++	-	-

Notes 1. Under usual field conditions, but which is modified by many factors.
2. *Trypanosoma brucei gambiense* and *T. brucei rhodesiense* cause human sleeping sickness in west and East Africa respectively and human animal reservoir, in which pathogenicity is low.
3. Mechanical transmission by bitting flies other than tsetse fly.
4. Transmission is venereal.
5. Rarely encountered.

Source: Connor (1991): Proceedings of the FAO panal experts Harare, Zimbabwe 24-26 June. pp. 1 - 38.

APPENDIX 11

GENERIC AND TRADE NAMES OF TRYPANOCIDES FOR THE TREATMENT AND PREVENTION OF ANIMAL TRYPANOSOMIASIS.

COMPOUND GENERIC NAME	TRADE NAME/ MANUFACTURER	ACTION	RANGE OF DOSAGE RATES mg/Kg.	ROUTE OF ADMINISTRATION	REMARKS
Diminazine aceturate	Berenil/ Hoechst AG Germany	T	3.5 - 7.0	IM, SC	Also babecidal, toxic to horses, donkeys, dogs and camels.
Homidium bromide	Ethidium/ CAMCO Animal Health,UK	T(P)	1.0	IM	-
Homidium chloride	Homidium/ Rhone-Merieux, France	T(P)	1.0	IM	-
Isometamidium	Samorin/ Rhone-Merieux, France	T/P	0.25-1.0	IM(IV)***	Toxic above 2mg/kg Highly irritating avoid SC administration
Isometamidium	Trypamidium/ Rhone-Merieux, France	T/P	0.25-1.0	IM(IV)***	-
Quinapyramine sulphate*	Trypacide/ Rhone-Merieux France	T	3.0-5.0	SC	Rest animal before and after treatment
Quinapyramine prosalt**	Trypacide/ Rhone-Merieux France	T/P	3.0-5.0	SC	Dosage calculated as sulphate.

* = Re-introduced in 1985 to treat early *T. evansi* infections.
** = Prosalt is a mixture of sulphate and chloride salts of Quinapyramine.
*** = Source: Dowler *et al.* (1989).
T = Therapeutic action; P= Prophylactic action; (P)= Short prophylactic activity
IM = Intramuscular; Iv= Intravenous; Sc= Subcutaneous.

SOURCE: Connor, R. J. (1991). Proceedings of the FAO panel of experts, Harare Zimbabwe. 24-26th June. pp. 1-38.

APPENDIX III

PCV VALUES FOR THE *T. vivax* INFECTED AND CONTROL GOATS

Case No.	PCV Values								
*571	30.8	28.0	22.0						
*567	28.0	27.0	24.0	22.0					
*566	29.0	27.0	20.0	18.0	20.5	19.0	17.0	17.5	
*5256	31.0	28.0	24.0	16.0	20.8	18.0	18.5	16.8	17.0
**561	30.5	30.0	28.0	30.0					
	0	2	4	6	10	12	14	16	18
	DAYS POST INFECTION								

* Infected with *T. vivax*

** Control