EFFECT OF LACTOSE- IN- SALINE INFUSION ON SOME SERUM BIOCHEMICAL PARAMETERS OF CATTLE INFECTED WITH *TRYPANOSOMA V1VAX*

BY

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BEING A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY MEDICINE

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DECLARATION

I hereby declare that the content herein is a record of my original research and has not been submitted for the award of a degree at any other university. Due credit has been given to the information used from both published and unpublished works of other researchers.

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CERTIFICATION

This thesis titled **"Effect of Lactose-in- saline infusion on some serum biochemical parameters of cattle infected with *Trypanosoma vivax*"** by **Kadima B. Kwem** meets the regulation governing the award of the degree of MSc in Veterinary Clinical Pathology of Ahmadu Bello University, and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

TO:

God Almighty , for His abundant mercies and love for me throughout this study
And all the kind - hearted people who continually encouraged me.

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My foremost gratitude to God Almighty who is knowledge itself, and endowed man with knowledge that we may understand Him more and more.

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A B S T R A C T

Thirty six (36) apparently healthy Zebu cattle in Zaria, were assayed for some serum biochemical parameters. The mean serum concentrations were as follows Na^+ , 140.03 ± 5.54 (mMol/1); $C1^-$, 98.67 ± 2.23(mMol/l); K^+,4.36 ± 0.40 (mMol/1); HCO,, 23.69± 2.69 (mMol/1); Glucose, 4.64± 5.20 (mMol/1); Bilirubin, 6.94± 3.22 (mMol/1); BUN, 5.66 ±2.11 (mMol/1); AST 46.96 ±11.25 (IU/1) and Albumin, 26.6 ±3.16 (g/1). The standard deviations were within explanable limits and the mean serum concentrations of these parameters reflects that the animals under investigation were apparently healthy.

A set of four 4 (Group B) young zebu cattle were infected with a Zaria strain *Trypanosoma vivax.* The serum biochemical parameters was investigated, serum Na^+ and CI^- levels significantly ($p<0.05$) increased following decreasing parasitaemia on days, 6, 7 and 8 post infection (p.i), while the greatest drop was on day 9 p.i to a point of hyponatremia and hypochloremia. Serum K^+values significantly ($p<0.05$) decreased during this period, while serum HCO'_3 showed significant ($p<0.005$) intermittent increases at the same period indicating a possible metabolic disturbance and / or kidney's inability to control the electrolyte changes. Serum bilirubin and Albumin levels significantly ($p<0.05$) increased following increasing parasitemia due to erythroclensis taking place and catabolic breakdown of proteins following fever of parasitemia. Serum glucose parasitemia significantly increased following decreasing parasitemia indicating that there is a relationship between glucose levels and parasitemia.

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CHAPTER ONE

INTRODUCTION

Trypanosomosis is a protozoan disease of wild, domesticated animals and man, caused by parasite of the genus Trypanosoma, transmitted in nature cyclically by tsetse flies (*Glossina sp*) and reduvid bugs (*Mastomes natelensis*). Mechanical transmission by biting flies (*Stomoxys, Tabanus and Liperosia*) is possible or coitus in case of Equine venereal trypanosomosis.

The disease is widely distributed in the world wherever the vector and susceptible host are found. The disease was initially thought to be confined to Africa (Hoare, 1972) where the main vector (tsetse fly) was mainly found. However, the disease has been reported in South America, Mauritius, West Indies and even outside the tsetse belt in Africa (*Losos, 1986, Soulsbly, 19986*).

African animal trypanosomosis is a major cause of economic losses in tropical Africa including Nigeria, causing deaths, stunted growth, debilitation, poor production, infertility and premature births (Unsworth, 1953: Maxie *et. al.*, 1979; Ileomobade *et. al.*, 1981; O'Hara *et al.*, 1987; Oga'a *et. al.*, 1993; Ogwu *et al.*, 1986, and Sekoni, 1992). This has been said to limit the number of cattle in Africa to about 20 million, whereas the carrying capacity is about 140 - 200 million (Service, 1992). Hence Africa produce seventy times less animal protein per hectare than Europe, due to trypanosomosis.

In Nigeria only about a fifth of its total land area of 920,000 sq. km is free of tsetse flies, consequently leading to overgrazing of these areas, and the high infection rates of 70% in cattle and almost 80% infectivity observed in sheep and

goats (kilickendrick and Godfrey, 1963 Godfrey *et. al.*, 1965, Yesufu and Mshelbwala, 1973).

The control and complete eradication of trypanosomosis has remained a major problem in tropical Africa especially Nigeria where the present control measures are vector control, chemoprophylaxis of susceptible host and chemotherapy of infected host. Vector control is mainly by deforestation, game destruction and use of insecticide, these methods are not only expensive, but also result in ecosystem destruction (Maclennan, 1970 Fiennes, 1974) while chemoprophylasis and chemotherapy is faced with drawbacks such as high cost of drugs, increasing development of resistance of parasite to existing drugs, and absence of new drugs to replace the old ones (Murray *et al.*, 1991). Hence the observation that about seven million cattle die annually from trypanosomosis throughout large portion of Sub - Saharan Africa (Engstler and schauer, 1994).

It is important therefore, that to improve the livestock industry and improve animal protein supply and consequently human health in Africa, Trypanosomosis has to be controlled, and if possible completely eradicated. These can be achieved by thorough understanding of the pathogenesis and pathology of the disease.

Infections with pathogenic trypanosomes (*T. vivax, T. congolense, T brucei, T.simiae, T. evansi; T. suis; T. uniforme, and T. equiperdum*) is generally characterised by fever, (Hudson, 1944) anemia, emaciation and stunted growth (Maxie *et. al.*, 1979), still birth, reduced milk yield and reduce gestation period (Ogwu *et. al*, 1986; Mwongela *et. al.*, 1981; Sekoni, 1992).

Research has established that anaemia is a major significant

pathological feature of both animal and human trypanosomosis (Fiennes, 1954; Mackenzie *et. al.*, 1978, Koboyashi *et. al.*,1976) The mechanism of anaemia in trypanosomosis therefore has been a subject of numerous investigations and theories have been put forward to explain the anaemia. Amongst these theories, Fiennes (1974) suggested bone marrow depression as the possible mechanism while *Valli et. al.*, (1978) suggested haemodilution as the possible mechanism, However with the discovery that *T. vivax* and *T.cruzi* produce sialdase (Esievo, 1979; 1983, Pereira, 1983) it has been suggested that sialidase cleaves bovine red blood cell sialic acid (Esievo *et. al.*, 1982) exposing antigenic sites which may induce production of antibody during the diseases, thus rendering the red cells suitable for phargocytosis by hepatic and splenic macrophages (Jancik *et. al.*, 1978).

The mechanism of the uptake of desialylated red blood cell by the hepatic and peritoneal macrophages (Kuster and Schauer, 1981) has been shown to be through the b-galactosyl specific lectin on macrophages in rabbits (Muller *et.al.*1981;Muller and Schauer, 1983) and in bovine *in vivo* (Ibrahim personal communication). Following these discoveries, Lactose a dissacharide of galactose and glucose has been infused in normal saline into bovine infected with *T.vivax* and the anaemia was ameniorated (Umar, 1997; Umar *et. al.*, 1997) presumably by binding through its galactosyl end on the b-galactosyl specific lectin on the macrophages.

Indeed a lot of work has been done on the parasite enzyme system, but Seed *et. al.*, (1985) noticed great metabolic disturbances in laboratory animals infected with *T. congolense*. It would therefore be interesting to find out what metabolic changes are produced during infections in cattle, in terms of the electrolyte and acid-base status of the animals, particularly since the lactose was infused into the

cattle in large volume of normal saline as this may alter or upset the electrolyte or acid-base balance.

The objective of this research, therefore is directed towards :

- Study of the normal serum biochemical parameters of young zebu cattle in Zaria.
- Evaluate the electrolyte alteration of young zebu cattle infected with *T. vivax*
- Determine the effect of lactose in saline infusion on electrolytes and serum biochemical parameters of young zebu cattle infected with T. vivax.

CHAPTER TWO

2.0.0 LITERATURE REVIEW

2:1:0 GEOGRAPHICAL DISTRIBUTION

Trypanosomes are transmitted in nature cyclically by tsetse flies, and reduvid bugs in case of chaga's disease, some are mechanically transmitted by biting flies and even by coitus in case of equine venereal trypanosomiasis.

Distribution of the disease therefore is worldwide, wherever the vectors and susceptible hosts are found. The presence of a particular trypanosome in an area, depends on the existence of its vector in that Area.

In African tsetse infest a major part of its land, about 7 million km2 of its grazing land is inaccessible to livestock for gazing (David-West, 1987). Pathogenic trypanosomes transmitted by tsetse flies are found mainly in Africa (Hoare, 1972). However from the confinement of these tsetse belts in Africa, infection by *T. vivax* has been reported in South America, Mauritus, West Indies and outside tsetse belts of Africa (Losos, 1986, Itard, 1989). These have been attributed to the ease of mechanical transmission of these tryponsome by biting flies.

T.equiperdum is found in the middle and far east, while *T.cruzi* is found mainly in south America, occupying an area from Argentina Northward and Central America (Soulsby, 1986).

2:2:0 HOST RANGE

All species of salivarian trypanosomes have a wide range of domesticated and wild hosts. Domesticated animals (cattle, horses, sheep, goats, pigs, camels, cats and dogs) are susceptible to infection by *T. brucei, T. congolense, T. evansi,* while

T. vivax also infect all domestic animals except dogs, cats, and pigs. While *T.equiperdum* infects mainly donkeys and horses and *T. suis* infect only pigs, but *T simiae* infects pigs, camels, sheep and goats (Kaggwa *et. al.*, 1985, Losos, 1986).

T. cruzi dose not occur in most domesticated animals except dogs, cats and rarely pigs, hence dogs have been implicated as carriers of *T. cruzi*, hence serve as reservours to human beings (Moh *et. al.*, 1978).

Chickens were initially thought to be resistant to pathogenic trypanosomes, but susceptibility to *T. brucei* and *T. rhodesiense* infection has been reported (Minter-Goedbloed., 1981).

Laboratory rodents have been reported to be susceptible to infection by *T. vivax* (Y58), a strain from West Africa (Leeflang *et. al.*, 1976a, b; DeGee *et al.*, 1976). Report have also shown trypanosomes in tissues and blood of healthy game animals (Burridge et. al., 1970; Hoare, 1972) and *T. brucei* was isolated from a sick zebra (McCulloch, 1967).

Host range, varries therefore with the species and strain of the trypanosome while some strains are infective to almost all domesticated animals only some are infective to both domesticated animals and wild animals.

2:3:0 TRYPANOSOMOSIS IN A SUSCEPTIBLE HOST

The degree of disease produced by pathogenic trypanosomes in a host is inflenced by several factors amongst which are, the host, environmental and parasitic factors, as detailed below.

2:3:1 HOST SUSCEPTIBILITY

The susceptibility of different animals species to different species of trypanosomes varies, while some animal species are readily susceptible, some are not. Reports have shown that susceptibility of the host is age and breed dependent (Robberts and Gay, 1973; Murray *et. al.*, 1991; Fiennes, 1970; Wellde *et. al.*, 1979) but Murrary and Morison (1978) maintain that susceptibilty is related to the host ability to limit the level of parasitemia along with the ability to control or even eliminate the parasite.

2:3:1:1 Breed Factor

Some breeds of domesticated animals have been observed to survive and are productive in tsetse infested areas, they are the humpless breeds of cattle (Ndama, Muturu and Keteku). (Robberts and Gay, 1973; Mortelmans and Kegeruku, 1976). and certain breeds of sheep and goats indigenous to east Africa are reported to be resistant to trypanosome infections (Griffin and Allonby, 1979), However variation in susceptibility of Nigerian breed of sheep (Maikaje *et. al.*, 1991) and goats (Anosa and Isoun, 1983) to trypanosome infections have been reported and certain strains of inbred mice have been reported to be trypanotolerant (Marrison *et. al.*, 1978).

Trypanotolerance is reported to be due to the ability of the host to control parasitaemia and development of anaemia (Murray *et. al.*, 1991), it is possible therefor that the mechanism of trypanotolerance might have evolved from the wild, due to continued exposure to the parasite, although also it is possible that game animals get infected with trypanosomes and die of the disease before discovery. However, wild animals have been reported to habour trypanosomes (Burridge *et al.*,

1970; Hoare 1972) and have been suggested to serve as the major reservour of pathogenic trypanosomes to domestic animals (Molyneux and Ashford, 1983).

2:3:1:2 Age Factor

The influence of age factor in the susceptibility of domestic animals to trypanosome infection varies from one animal species to another.

In cattle, calves have been reported to be relatively resistant to *T. congolense* infection (Fiennes, 1970; Wellde *et al.*, 1979), while young trypanotolerant breed (Ndama), infection with *T. vivax*, showed severe disease condition, increase herd mortality rate, reduced weight gain and birth weight (Otesile *et. al.*, 1990). Similarly young sheep and goats within the age of 2 - 64 days are more susceptible to *T.congolense* infections than adult sheep and goats (Griffin and Allonby, 1981).

In camels, increasing pathological prevelance of *T. evansi* infections is almost non-existence in the young less than one year old. And human infections with *T.cruzi* have been noticed to cause severe disorder, particularly more severe in children, with higher mortality than in adults (Itard, 1989).

2:3:2 PARASITE STRAIN/SPECIES

Infection of different hosts by different species or strains of trypanosomes results in different types of disease syndromes of varrying severity (Stephen, 1970). *T. congolense* infection in cattle has been reported to produce severe disease, while its infection in pigs results in mild or symptomless infection (Ilemobade and Balogun, 1981; Stephen, 1966a). Furthermore, Godfrey (1961), reported that the Nigerian strain of *T. congolense* has low infectivity, pathogenicity and parasitemia, compared

to the virulent east African strain, that causes more severe and acute disease in ruminants (Losos, 1986).

T. vivax infection of goats results in a more severe disease than infection in sheep (Anosa and Isoun, 1979; 1980). The West African strain *T. vivax* is said to be more virulent than the East African strain (Fairbrain, 1953). *T. brucei* has been reported to cause severe disease in dogs, horses , donkeys (Losos and Ikede, 1972 Ikede *et. al.*, 1977) in sheep and goats (Losos and Ikede, 1975), but pathogenicity in cattle has not been settled. However it is reported to cause mild or asymptomatic desease in cattle (Losos, 1986).

2:4:0 PATHOGENESIS AND PATHOLOGY OF TRYPANOSOMOSIS IN CATTLE

Trypanosome infections are capable of evoking a range of responses in susceptible hosts. The disease produce ranges from acute, subacute, chronic to asumptomatic forms (Hudson, 1944). However the disease has been reported to be generally charactertised by bouts of fever (Hudson, 1944; Valli *et al*, 1979; Anosa *et. al.*, 1980) weight loss decreased milk yield, abortion, reduced production and reduced gestation period (Mwongela *et al.*, 1981; Ogwu *et al.*, 1986)

2:4:1 Pyrexia

Pyexia has been observed to be one of the early clinical signs of trypanosomosis (Hudson, 1944; Valli *et al.*, 1978; Griffin and Allonby, 1981). The rise in body temperature usually marks the onset of parasitemia (Luckins, 1976). High rise in body temperature has been associated with high level parasitemia in acute

severe disease of cattle infected with *T. vivax* (Losos, 1979; Murray and Morrison, 1979a), but Stephen, (1986) maintains that the extent of rise of body temperature may not necessarily reflect the degree of parasitemia. However it has been observed that following the fall in body temperature, parasitemia also falls, but thereafter periodic waves of parasitemia succeed each other at few days intervals with the temperature not getting as high as the first peak (Edward *et al.*, 1956; Godfrey, 1961; Maxie *et al.*, 1976; Esievo *et al.*, 1982). The temperature pattern of trypanosome infection therefore is said to be relapsing type, rising and falling with the parasitemic wave (Luckins, 1976; Stephen, 1986).

2:4:2 Parasitemia

The appearance of trypanosomes in blood stream of susceptible host following cyclical or needle transmission is usually preceeded by a prepatent period, which may last from few days to weeks. Levels of parasites in the blood have been observed to increase with increase in body temperature to a peak, then followed by fall in parasitemia and thereafter periodic waves succeed each other (Godfrey, 1961; Assoku, 1975; Maxie *et al.*, 1979). Each succeeding wave represents the multiplication of a new antigenic population, while the desceding phase represents the destruction of some members of the parasite by host immune responses (Lumsden, 1972; Murray and Morrison, 1978), but infections by most pathogenic trypanosomes result in tissue invasion and localization in the organs (Godwin, 1970, Losos and Ikede, 1970 Morales and Carreno, 1976; Yutuc and Sher, 1949) therefore changes in parasitemic wave may not necessarily be due to host immune response, but probably due to migration of parasites between blood and solid tissues.

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2:4:3 Anaemia

Aneamia is clinical feature of both human and animal trypanosomosis (Fiennes, 1953; Holmes and Mamo, 1975; Koboyashi *et al.*, 1976; Mackenzie *et al.*, 1973) and has been established as very signficant pathological feature in animal trypanosomiasis caused by *T. vivax* and *T. congolense* infections (Fiennes, 1970; Losos and Ikede 1972).

The onset of anaemia has been observed to relate with the onset of parasitemia in animals (Fiennes, 1970, Roberts and Gray, 1973;Maxie *et al.*,1970a,b;) and the anaemia is manifested by decreased packed cell volume (PCV), decreased haemoglobin concentration, and reduced red blood cell counts at the peak of parasitemia, zebu cattle have been observed to develop greater parasitemia than Ndama cattle, and the resultant anaemia more severe in zebu, suggesting that Ndama cattle would need a higher parasitemia to attain the same level of anaemia as in zebu cattle (Dargie *et al.*, 1979). Similarly it was observed that in *T. vivax* infected cattle (Saror, 1979) and in *T. brucei, T.congolense* and *T. vivax* infected mice (Reather, 1971 ., Morrison *et al.*, 1978) a single intensive wave of parasitemia resulted in severe anaemia. It seems therefore that anaemia development depend on the intensity and duration of the initial peak of parasitemia.

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2:4:3:1 Mechanism Of Anaemia In Trypanosomosis

The mechanism of anaemia in trypanosomosis has been a subject of many investigations and many theories have been put forward. Amongst the earliest observations was bone marrow depression in *T. congolense* infected cattle (Fiennes, 1954, Naylor, 1971b; Losos and Ikede, 1972) and in *T. brucei* infected rabbits

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(Boycott and Price-Jones, 1913). However following these reports has been the observation of active erythropiesis in *T. brucei* infection of rats (Jenings. 1974, Murrary *et al.*, 1974). *T. evansi* infection of rats (Assoku, 1975). *T. vivax* infected goats (Saror, 1980) and cattle (Ingh *et. al.*, 1976a) and *T. congolense* infected sheep (Mackenzie and Cruikshank, 1973).

The variations and inconsistency of these findings have been suggested to be probably due to variations and inadequacies of technics used (Dargie *et al.*, 1979a; Mamo and Tartour and Idris, 1973 ;Holmes, 1975; Valli *et al* , 1978b, Maxie *et. al.*, 1979;) However Dargies et al (1979a) following his observation of the response of Ndama cattle and zebu cattle to *T. congolense* infection concluded that the resistance of Ndama cattle to development of anaemia lies in the control of parasitemia by the host, rather than in more efficient erythropoietic responses suggesting then that even if dyshaemopoiesis contributes to the development of anaemia during trypanosomosis, its effect is mild to moderate in cattle.

The anaemia in cattle infected with trypanosomes has been associated with antigen-antibody complexes and complements on the surfaces of erythrocytes (Koboyashi *et. al.*, 1976; Assoku, 1975; Woo and Koboyashi; 1978) following the observation of antigen-antibody complexes on the surface of red blood cells of calves infected with *T. congolense* (Koboyashi *et. al.*, 1976), and the observation of moderately severe anaemia in rats intravenously injected with *T. evansi* antigen (Assoku, 1975).

Jennings *et. al.*, (1974) however postulated that erythrophagocytosis is the mechanism by which red blood cells are sequestrated. In this vein Mackenzie *et. al;*., (1978) demonstrated trypanosoma material on sheep Rbc by indirect flourescent

antibody test, and concluded that phargocytosis of sheep erythrocytes was due to the coating of the surface of red blood cells by antigens. Also anti-red blood cell antibody was generated in *T. brucei* infected mice (Anosa and Obi, 1980) and in a haemorrhagic type of bovine *T. vivax* infection (Assoku and Gardiner, 1989). Since immune complexes responses are not normally significant earlier than day seven after infection, any erythrophagocytosis observed earlier might occur from some other reasons than immune complexes.

However with the discovery that the enzyme sialidase is produced by *T. Vivax* (Esievo, 1979; 1981) *T. cruzi* and *T. rangeli* (Pereira, 1983; Pereira *et. al.*, 1984; Reuters *et. al.*, 1984). It was postulated that sialidase cleaves red blood cell surface sialic acid during bovine trypanosomosis (Esievo *et. al.*, 1982) and new antigenic sites might have been exposed on the affected erythrocyte as earlier observed by Pirofsky (1969). Katz *et. al.*, (1977) observed that during the course of disease, antibodies may then be produced against these new sites on the red blood cell surface rendering them suitable for erythrophagocytosis (Esievo and Saror, 1991) thus suggesting that sialidase may be playing an important role in the early anaemia observed in the disease.

Erythrophagocytosis therefore may be responsible for the early anaemia observed in trypanosomiasis. The mechanism which may be by the recognition and sequestration of sialidase treated Rbc by phagocytic cells of liver and spleen (Jancik *et. al.*, 1978), since the loss of animal Rbc sialic acid and accelerated death of desialylated Rbc by sequestration was achieved using *Vibrio cholerae* sialidase in vitro (Durocher *et. al.*, 1975). Following these observations, Kuster and Schauer. (1981) reported that desialylated Rbc are removed from circulation through

phargocytosis by rat peritoneal macrophages by binding via a β-d-galactosyl specific lectin on the marcrophage surface, further experiment revealed that in rabbits, a b-galagctosyl specific lectin seems to be involved in the mechanism of sequestration in vivo (Muller *et. al.*, 1981; Muller and Schauer; 1983). Thus sialidase treatment exposes b-galactose residue of the Rbc membrane and interaction of these cells with b-galactose specific lectin on the liver and spleen macrophages leads to phagocytosis of the desialylated Rbc (Muller *et. al.*, 1983a,b).

In an attempt to find out if phargocytosis of erythrocytes, due to the removal of sialic acid during *T. vivax* infection of cattle occurs and if inhibition of erythrophagositosis and perhaps prolongation of Rbc-life span can be achieved, Lactose a dissacharide of galactose and glucose was infused in normal saline into bovine experimentally infected with *T. vivax* and the anaemia was ameniorated (Esievo *et. al.*, 1994; Umar *et al.*, 1992) presumably by Lactose attaching through the galactosyl end of the b-galactosyl specific receptors lectin in Kuffer cells of liver and splenic macrophages of the infected bovine

The effect of large volume of lactose in normal saline solution needs to be investigated in relation to body electrolytes metabolic changes in the host.

2:5:0 SERUM BIOCHEMICAL CHANGES DURING TRYPANOSOMOSIS

Serum biochemical changes has been a subject of many studies, with the view to understand the pathogenesis and pathological changes of trypanosome infected host.

Anosa (1988) noted that despite the variation in hosts, and parasite species,

the severity of hematological and biochemical changes associated with the various host parasite combination is determined by the level of parasitemia which develops during the early phase of the infection.

2 :5 :1 Serum Proteins

Total protein levels has been reported to increase in *T. vivax* infected sheep and goats (Clarkson, 1968; Anosa *et. al.*, 1978), *T. brucei* infected sheep and goats (Ogunsami *et al.*, 1994; Igbokwe *et. al.*, 1992). While reduced values were reported in *T. rhodesiense* infections of monkeys (Sadun *et. al.*, 1973) , and *T. brucei* infection of dogs (Kaggwa *et. al.*, 1984) but normal total protein levels were reported in *T. rhodesiense* infected monkeys and mice (Moon *et. al.*, 1968; Smithers *et. al.*, 1956), in *T. evansi* infected buffalo calves (Kathiria *et. al.*, 1985), and camel (Singh and Guar, 1983), and in *T. brucei* infection of dogs (Omotainse *et. al.*, 1994).

The reason for the variation in the above report is not very clear, but plasma volume changes during trypanosomiasis have been reported (Clarkson *et. al.*, 1975; Anosa and Isoun, 1977) therefore the decreased values may be due to expansion of plasma volume as a physiological event evoked by the host need to maintain constant blood cell mass, thereby resulting in dilution of dissolved content of plasma including proteins. However Anosa (1988) noted that the effect of trypanosomiasis on the total plasma protein levels depends on the species of trypanosome, animal host, and other factors such as bred and nutritional status. As indicated in work on deer mice infected with *T. brucei*, infected mice that became anaemic had normal total protein levels, while the more resistant group had elevated serum proteins level (Anosa *et. al.*, 1983), indicating that the severity of the disease influences the total protein levels.

Fractionilization of plasma proteins has shown that serum albumin levels was depress in *T. brucei* infected sheep (Ogunsami *et. al.*, 1994) mice (Anosa and Kaneko, 1983b) and dogs (Kaggwa *et. al.*, 1984). *T. vivax* infected sheep and goats (Clarkson, 1968, Anosa and Isoun, 1976), while the serum globulin levels were elevated in *T. brucei* infected mice (Anosa and Kaneko, 1988b), sheep and goats (Ogunsami *et. al.*, 1994; Igbokwe *et. al.*, 1992), resulting in low albumin/globulin ration (Anosa and Kaneko, 1983b; Ikejiani, 1946; Smithers *et. al.*, 1959; Igbokwe *et al.*, 1992). Globulins are actively involved in immunogen synthesis the increase may indicate that immunological responses are taking place during infections. Further evidence for the immunologiocal responses during disease includes the observation of elevated IgM (Clarkson *et. al.*, 1975; Koboyashi and Tizard, 1976; Moulton and Sollod, 1979; Luckins and Gray, 1978; Table *et. al.*, 1980) and complement fragments on cell surfaces (Koboyashi and Tizard, 1976) during the disease.

2:5:2: Serum Glycoproteins

Plasma concentrations of glycoproteins (hexose, hexamine, sialic acid and seromucoid fractions) increased in cattle infected with *T. brucei* and *T. vivax*, during the first few weeks of infection when parasites became scanty (Magaji, 1975).

Sialic acid (glycoprotein) has been shown to occur in varying concentrations, as a terminal structure of glycoproteins of human and animal erythrocytes (Yaari, 1969; Esievo *et al.*, 1983; Onyemelukwe *et. al.*, 1987). The significance of sialic acid in animal trypanosomiasis has been reported. Banks (1979; 1980) reported the attachment of *T. congolense* to cattle red blood cell sialic acid causing erythrocytic

injuries, while Esievo (1979; 1983) reported the production of sialic acid cleaving enzyme sialidase by *T. vivax*, as Pereira (1983) reported same for *T. cruzi*, thereby, suggesting that the development of anaemia in zebu cattle has been associated with red blood cell surface sialic acid concentration during parasitemia of experimental *T. vivax* infection; presumably by trypanosome producing sialidase which cleaves surface sialic acid thus rendering them more prone to sequestration by macrophages of Reticuloendothelial system (Esievo *et. al.*, 1982).

2:5:3 Serum Glucose

Serum glucose levels have been reported to decrease in acute phase of *T. evansi* infected buffalo calves, and donkeys (Singh and Guar, 1983; Jani *et al.*, 1992), in paracute *T. simiae* infected pigs and *T. rhodesiense* infected mice (Moon *et. al.*, 1968) and in experimental infected *T. simiae* of sheep (Joshua *et. al.*, 1985). In cattle decreased serum glucose levels accured at periods of high parasitemia with acute *T. vivax* and *T. congolense* infections (Hudson, 1944) , but not in chronic infections. No significant changes in serum glucose levels have been reported in *T. evansi* infected buffalo calves (Singh *et al.*, 1983; Kathriria *et al.*, 1985) at the chronic phase of the infection and also in chronic infections in monkeys (Sadun *et. al.*, 1`973) and Man (Borret-Connor *et. al.*, 1973). It seems therefore that decreases in serum glucose level occur at periods of high parasitemia, at the early part of infection, may be the large number of parasites, associated with high parasitemia utilize glucose for their metabolism, since trypanosomes are known to require glycolytic pathway as an exclusive energy pathway, invading host tissues and exploiting the carbohydrate reserves to support its existence in the host (Opperdoes

and Borst, 1977). However, Von Brand (1973) maintained that the decreased glucose levels is more due to breakdown in hepatic and endocrine mechanisms controlling the carbohydrate metabolism, than a direct result of massive consumption of glucose by the trypanosomes. The question one may ask is, How early is organ and tissue damage observed in acute trypanosoma infections?

2:5:4 Serum Urea Nitrogen (Bun)

Serum urea nitrogen has been observed to increase in cattle infected with *T. vivax* (Hudson, 1944; Isoun *et al.*, 1979), *T. rhodesiense* infected monkeys (Sadun *et. al.*, 1973) and mice (Moon *et. al.*, 1973), in *T. evansi* infected donkeys (Jani *et al.*, 1992) and *T. brucei* infected sheep (Ogunsami *et. al.*, 1994), and dogs (Omotainse *et. al.*, 1994).

In cattle, the increase in urea nitrogen levels were observed at periods of high parasitemia in acute infections, but terminally in chronic infections; One may speculate then that the increases in acute infection could be due to feverish conditions associated with high parasitemia in trypanosomosis (Losos, 1979a; Murray and Morrison, 1979a), since catabolic breakdown of proteins and tissue leading to increase in blood urea nitrogen is associated with feverish conditions (Coles, 1986). While the increases in BUN associated with chronic infections might have been due to kidney damage during the disease, as glomerulonephritis is a feature of chronic trypanosomosis (Tizard and Soltys, 1971).

2.5.5 Serum Bilirubin

A consistent finding in the infection of animals and humans with pathogenic

trypanosomes is accelerated reduction in red blood cell half life and its consequent destruction by erythrophagocytosis (Anosa and Isoun, 1983; Anosa *et. al*, 1977; Ikede and Losos, 1972; Fiennes, 1954; Losos *et. al*, 1973; Mackenzie *et al*, 1978; Mackenzie and Cruikshank, 1973; Jennings *et. al*, 1974; Banks, 1979; 1980; Esievo, 1980).However normal bilirubin levels were recorded in *T.brucei* infected mice, monkeys and man (Moon *et. al*. 1968, Sadun *et. al*, 1973; Borret-connor *et. al*, 1973). These normal values appear to be inconsistent with the increase in red blood cell destruction during trypanosomosis, however, it is noteworthy that normal bilirubin levels could exist in hemolytic diseases, since the liver functional reserve can be mobilised in such a condition to conjugate and excrete excess bilirubin produced (Coles, 1986). This might be happening in trypanosomosis since elevated levels of bilirubin was noticed in acute infections of cattle with *T. congolense*, but not in chronic infections (Fiennes, 1954).

2.5.6 Serum Enzymes

Serum transaminases have been measured during trypanosoma infections in relation to parasites effect on host tissue. This is because some enzymes are tissue specific. Increased levels of serum GOT and GPT has been observed in *T. rhodesiense* infected mice, monkeys and man (Moon *et. al*, 1968; Sadun *et. al*,, 1973; Borret-connor *et. al*, 1973), in *T.vivax* infected cattle and sheep, (Gray, 1963), and *T.evansi* infected buffalo calves (Singh and Guar, 1983). Also increases were observed in *T. brucei* infected dogs early in the disease but decline as a disease progressed (Kaggwa *et. al*, 1984).

The increases observed during the disease corresponded with the early phase

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of the disease at periods of high parasitemia, days too short for any severe tissue damage to have taken place, considering that increases are associated with tissue or organ damage, the increase early may have been due to the parasites releasing metabolic by-product and not necessarily due to any organ damage. In support of this observation high levels of serum GOT and GPT in suspension of living trypanosomes and their homogenates (Stephen and Gray, 1960).

2.5.7 Serum Cations and Anions.

The major cations and anions involve in electrolyte balance and acid base status of animals are Sodium(Na^+) potassium (K^+), Chloride (Cl^-) and Bicarbonate (HCO_3-). (Coles 1986). Study of these electrolytes has been carried out in trypanosomiasis but not in relation to the acid-base status of the infected animals. Blood levels of Na^+ were observed to be normal in acute T.rhodesiense infected mice and man (Moon *et. al*, 1968, Borret-connor *et. al*, 1973), in *T.evansi* infected Camel (Raisinghari *et. al*,, 1979) and *T.brucei* infected sheep (Ogunsami *et. al*,, 1994).

Serum levels of K^+ were normal in *T.rhodesiense* infected mice (Moon *et. al*, 1968), but elevated in *T.brucei* infected sheep and *T. equiperdum* infected rats (Ogunsami *et. al*,, 1994, Ikejiani, 1946). The increases in blood potassium levels corresponded with decrease in red blood cell values, hence was attributed to hemolysis during the disease, because potassium is predominant and intracellular electrolyte, therefore, red blood cell lysis may be releasing it into circulation hence the increase observed. However, it has been suggested that the increases in potassium levels during the disease might have been due to acidosis associated with splenomegaly (Sadum *et al* , 1973) and myocarditis seen in trypanosomosis. A

probable hepatic and or renal malfunction has been suggested (Ogunsami *et. al*, 1994). Increases in HCO_3^- is reported in *T.brucei* infected sheep (Ogunsami *et. al*,, 1994), while a non-significant significant change was observed in *T.brucei* infected goats (Igbokwe *et. al*, 1992).

The variation in these electrolytes indicate profound disturbances of physiological mechanism generally associated with impaired function of many organs, that control their regulations especially the kidney. Many workers therefore have associated the observed electrolyte changes to tissue and kidney damage. (Fiennes , 1946; Ikejiani, 1946; Losos and Ikede, 1972; Sadun, *et al*, 1973; Murray and Marrison, 1978; Ogunsanmi *et al*, 1994) . The question one may ask is how early is tissue or organ damage during trypanosomosis? It is possible that the electrolyte changes observed are by a mechanism other than tissue or organ damage; the observation that the enzyme Na^+-K^+ Atpase which is chiefly involved in the maintenance of ionic balance in cells (Sweadner *et al*, 1980) and found in high concentration in the kidney (Delvin, 1986) decreases with advancing paracetamia in rats infected with *T. congolense* (Nok *et al*, 1992) may be suggesting modulation of the Na-pump leading to ineffective ionic balance early in the infection.

The question one may further ask is, how do these electrolyte changes affect the host metabolic status and consequently the disease in a susceptible host.?

2.6.0 EFFECT OF TRYPANOSOMOSIS ON HOST METABOLISM

Trypanosome infection have been reported to elicit several changes in the host metabolicsystem (Seed *et. al.*, 1985), these changes have been studied in relation to tissue and organ damage caused by the parasite presence in the host, hence

metabolism changes in the host has mainly been associated with acidosis due to splenomegaly which is a common feature in animal trypanosomiasis (Sadun *et. al.*, 1973; Losos and Ikede, 1972). However, Murray, (1974) associated the acidosis to myocarditis leading to congestive heart failure, the accompanying decrease in cardiac output, leading to Na+ and water retension by the kidneys.

The kidneys as the major organ involved in regulatory and electrolyte balance by its endocrine system (Coles, 1986) and has been incriminated by many workers to be responsible for the metabolic disturbances that have been observed (Fiennes, 1946; Ikejiani, 1946; Ogunsami *et. al.*, 1994; Nok *et. al.*, 1992). Nok *et. al.* (1992) observed modification of Na-pump in *T. congolense* infected rats and suggested that to likely be responsible for the ineffective ionic balances during trypanosomosis that been reported.

Generally, the observations therefore suggest that infection with trypanosomes affect the electrolytes and consequently the acid-base status of animals infected. The extent of these changes is not yet ascertained, but in an attempt to find out if the erythrophagocytosis is due to the removal of sialic acid during *T. vivax* infection of cattle occurs through exposure of b-galactosyl specific lectin on macrophages. Umar *et. al.*, (1997) infused lactose a dissacharide of glucose and galactose in normal saline as a function of the animals weight, their blood volume being assumed as 6-7% of their body weight. The question is, What is the effect of such quantity of saline solution on the electrolyte balance of the animal and consequently is acid- base status?.

It is necessary for proper chemotherapy that side effect should be reduce to minimal, if not completely removed. Therefore the basis for this research is to

understand the electrolyte and acid-base status of animals infected with *T.vivax* and also infused with lactose in saline solution, as the infusion of large quantity of saline solution may be causing some electrolytes changes in the animal infected and infused.

CHAPTER THREE

3.0.0 SERUM BIOCHEMICAL PARAMETERS OF APPARENTLY HEALTHY YOUNG ZEBU CATTLE IN ZARIA.

3.1.0 INTRODUCTION

Normal blood and serum biochemical parameters in Zebu cattle and their cross breeds had bee reported in Nigeria (Garner, 1952; Oduye and Fansami, 1971; Saror and Coles, 1973; Akerejola *et. al.*, 1980; Oyedipe *et. al.*, 1984). Prior to this normal values were extrapolated from those of animals in the temperate environments.

In Nigeria however, interbreed differences in serum electrolytes values in cattle (Oduye and Fansami, 1971) has been reported, and basic differences in serum biochemical values between Zebu and Friesian cattle had also been reported (Akerejola *et. al.*., 1980; Olusanya, 1976). This led to the suggestion that breed and environment play a significant role in using blood and serum biochemical parameters in assessing the significance of disease in the tropics.

It is therefore necessary to establish baseline of serum biochemical parameters levels for our indigenous zebu cattle to help in assessing the significance of the electrolyte of the electrolyte balance and acid-base status of animals during disease in our environment.

3. 2 .0 MATERIALS AND METHOD

3 .2 .1 Experimental Animals

Thirty-six (36) young zebu cattle of between the age of 12 - 18 months randomly selected from herds around Zaria. These animals were both females and

males and were manage semiintensively in their various farms. They had no previous history of exposure to trypanosome infection . They were already acclimatized, since they were resident around Zaria.

3. 2. 2. Sample Collection

The selected animals were not given any special treatment before sampling. Five milliliters (5ml) of blood were collected early in the morning about 8.00am local time from each animal by jugular venipuncture and placed into clean vaccutainers without any anticoagulant. Serum was harvested within one hour of sample collection by centrifugal at 200g/15min. Serum was immediately stored at -20°C prior to analysis.

3 .2 .3. Sample Analysis

Serum sodium (Na^+) and potassium (K^+) concentration were analyzed by flame photometry, Serum chloride (CL^-) values was by the method of Schales to Schales, (1941) as out lined by Coles (1974), Serum bicarbonate (HCO_3-) was analyzed by Titrimetric method out lined in Cheesebrough, (1991), while serum albumin was by Buiret method as out lined by Cheesebrough, (1991). Serum urea nitrogen (BUN) was by the diacetyl monoxime method as out lined by Strove and Makarova, (1989) while serum glucose was by oxidation method as out lined by Cheesebrough, (1991) and serum Bilirubin values were analysed by the method Jendrassik *et. al.*,(1938) as out lined by Cheesebrough, (1991). Serum enzyme aspartate aminotransferase (AST) was analysed by Reitman and Frankel method (1957). For brief description of each method see Appendix-1.

3.30 RESULTS.

The means and standard deviations (x ± S.D) of all the biochemical parameters of the animals sampled are indicated in Table-1

TABLE -1: MEAN SERUM BIOCHEMICAL PARAMETERS OF THIRTY SIX (36) APPARENTLY HEALTHY YOUNG ZEBU CATTLE IN ZARIA

	Sodium Na^+ (mMol/1)	Chlorine Cl^- (mMol/1)	Potassium K^+ (mMol/1)	Bicarbonate HCO_3- (mMol/1)	Blood Urea Nitrogen BUN (mMol/1)	Glucose Glu (mMol/1)	Aspartate Amino Transferase AST (I.U/L)	Albumin Alb (g/l)	Bilirubin Bil (μMol/1)
Mean and Standard Deviation (x ± S.D)	140.03±5.54	98.67±2.33	4.36±0.40	23.69±2.69	5.66±2.11	4.64±5.20	46.90±11.25	26.6±3.16	6.94±3.22

3. 4. 0. DISCUSSION.

The mean serum Na^+ was 140.03± 5.54mMol/l and mean K^+ value was 4.36mMol/l, these values were within the range observed by Akerejola *et. al*, (1980);Oduye and Fansami(1971); Coles(1974) and Esievo and Moore (1990) while the mean Cl^- value of 98.67±2.33mmol//l was within the range observed in high producing Holstein-Friesian cattle and also in Zebu cattle (Esievo and Moore,1990; Akerejola *et.al.*,1980) Because Na^+ is mainly extracellular, and is actively transported Cl^- is excreted and distributed passively according to the electrical gradients established by the active transport of Na^+(Coles,1986). It is therefore not surprising that it has been observed that there is reciprocity in values of Na^+ and Cl^- between breeds, the lower the Cl^- value the higher the Na^+, and similarly the correlation between K^+ and Cl^- in relation to the breed (Akerejola *et.al.*,1980).

The mean serum HCO_3^- value was 23.69 ± 2.69mMol/l, and falls within the range observed by Coles(1986), but a further interpretation in relation to acid-base status of these animals would have been more meaningful in combination with carbon dioxide, and hydrogen ion (H^+) determination, but the latter were not determined during this investigation.

The mean Serum Urea Nitrogen (BUN) was 5.66±2.11mMol/l, this value was lower than that observed by Akerejola *et. al,*. (1980), Coles(1974) Saror and Coles(1973),but was within the range observed in medium and low protein diet zebu (Oyedipe *et.al.*,1984). While the mean serum albumin value was 26.6±3.16g/l value lower than that observed by Akerejola *et. al*,(1980); Saror and Coles(1973), but was within the range observed by Oduye and Fansami (1971),Ross,(1960) and Oyedipe *et. al.* (1984).The variations could be ascribed to the influence of nutritional,

immunological, physiological and pathological conditions on plasma proteins and one of these factors might have affected the values observed in this study.

The mean Serum glucose values was values was 4.64 ± 5.20mMol/l, and agrees with the normal value recorded in Coles (1986), suggesting normal glucose metabolism in the animal in this study. However, the greater standard deviation observed, was due to some animals having greater glucose value than others, thus large standard deviation, this is expected considering that each of the animal under study had different metabolic activity.

Mean Serum aspatate amino transferase (AST) was 46.9 ±11.25 IU/L and the mean serum bilirubin value was 6.9 ±3.22 μ Mol/l and these values are within normal values observed by Coles (1986) indicating the absence of tissue damage in these animals and normal liver function.

In general, the standard deviations during this study were within explainable limits and the mean concentration of all the biochemical parameters under investigation reflected that the animals were apparently healthy, therefore mean values significantly above or below these concentration in table 1 would point to a derrangement from the normal electrolytes and other biochemical parameters levels of zebu cattle in Zaria area.

CHAPTER FOUR

4.0.0 THE EFFECT OF LACTOSE IN SALINE INFUSION ON SERUM ELECTROLYTES ALTERATION OF CATTLE INFECTED WITH *TRYPANOSOMA VIVAX*

4.1.0 INTRODUCTION

It has been over a decade ago the production of potent enzyme antigen, sialidase had been ascribed to *Trypanosoma vivax* (Esievo,1979;1983), *T.cruzi* (Pereira,1983) and *T.rangeli* (Reuters *et. al.*,1984). The manipulation of the hosts sialoglyco-conjugates by this potent enzyme were incriminated in the pathogenesis of cattle trypanosomosis (Esievo *et. al.*, 1982) and human chagas disease (Pereira and Hoff, 1986). The various biochemical differences existing in the erythrocytes siaglyconjugates of the trypanotolerant Ndama and the trypanosusceptible zebu cattle (Esievo *et. al.*, 1986; 1990; Shugaba *et. al.*, 1994) gave further credence to the relative trypanotolerance of the former and the significance of the trypanosome sialidases and the host sialoglyconjugates in the pathophysiology of the anaemia of the bovine trypanosomosis as earlier reviewed (Esievo and Saror, 1991).

Recent studies have shown that the sialidase cleavage of erythrocytes sialic acids during the course of cattle trypanosomosis (Esievo *et. al.*, 1982) led to the exposure of the permulltimate ß-galactosyl residues on the erythrocyte membrane (Esievo *et. al.* 1994; Omage *et. al.*, 1997) and enhanced activities of the Kuffer cells surface lectins which bound to the desialylated erythrocyte membrane galactose, hence phagocytosis and sequestration of the desialylated red cells (Ibrahim *et. al.*, 1997). These latter processes can be inhibited by lactose thus supporting lactose as

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an incipient to a trypanocidal agent. For this reason, the kinetics of intravenously infused lactose in cattle (Umar et. al., 1997a) and the effect of *T.vivax* infection on the lactose kinetics were established (Umar et. al., 1997b, Umar, 1997). The volume of lactose in normal saline infused , made it imperative to investigate the effects of the infusion on the electrolytes in cattle *T. vivax* infection, as reported herein.

4.2.0 MATERIALS AND METHODS

4.2.1 Experimental Animals

Twelve male Zebu (Sokoto Gudali) calves aged 12-18 months, weighing between 80-90kg and indigenous to Nigeria, were purchased from Talata Mafara (Sokoto State), an area known to be tsetse free. These animals were housed within the premises of the faculty of veterinary medicine, Ahmadu Bello University Zaria, and screened for helminths, haemo and ecto-parasites. They were dewormed with thiabendazole (Merck, Sharp and Dohme Ltd, UK), sprayed against ticks at weekly intervals with chlorphenviphos (Pfisona®, Pfizer Nig.Ltd) and vaccinated against Anthrax, Balckquarter, Contagious Bovine Pleuropneumonia and Rinderpest.

The animals were fed with hay supplemented with a mixture of cotton seed cake and wheat offals. Water and salt licks were supplied ad lib. The animals were allowed to acclimatize for about a month before the commencement of the experiment.

4.2.2 Parasites and Infection

A stock of *Trypanosoma vivax* was isolated from a pregnant cow with a pure natural infection and identified. Two millimeters (2ml) of the infected blood was used

to infect a clean Sahel brown goat which served as donor animal for the experimental calves, after the goat had developed massive first peak parasitemia the twelve calves were divided in 3 groups of four each marked A,B & C and housed separately .

Animals in group A served as uninfected control while groups B and C animals were infected with five millimeters of infected donor goats blood, containing about 11.0 x 10^6 trypanosomes by jugular venipuncture.

4.2.3 Lactose Preparation / Infusion

Lactose solution for infusion was prepared in a physiological saline (0.9%Nacl.). A 50% solution was prepared by warming and sterilized at 15/bs/sq in pressure for 5 minutes. The solution was cooled at room temperature slowly. The solution was warmed to 37°C before infusion into calves.

Each of the calves in group C were given lactose in normal saline solution intravenously at the dose rate of 0.5g/kg body weight daily at the rate of 18ml/min. The lactose was given as a function of the animals weight, their blood volume being about 6-7% their body weights as outlined by Umar *et. al.*(1997a,b)

4. 2. 4 Sample Collection

Five millimeters (5ml) of blood were collected daily from the cattle in groups A, B and C through the jugular vein into vacutainers with Ethylene Diaminotera-Acetate (EDTA.) as anticoagulant for routine haematology and estimation of parasites. Another set of 5ml of blood was also collected from groups A, B and C animals into vacutainer without anticoagulant; Serum was harvested from the clotted blood within one hour of collection by centrifugation at 200g/15min and immediately

stored at -20oC prior to analysis.

4 .2 .5 Estimation of Parasitemia

Trypanosomes were detected in the blood sample by the micro-haematocrit centrifuge technique. The trypanosomes seen in the buffy coat area of the haematocrit were counted and parasimtemia scores were given as, +1 (occasional trypanosome seen), 2+ (1-2 trypanosomes seen), 3+ (3-20 trypanosomes) and 4+ (>20 trypanosomes seen) the parasite score method as described by Woo. (1969).

4. 2 .6 Sample Analysis

Packed cell volume (PCV) was determined by standard microhaematocrit method while serum electrolytes were assayed by the methods mentioned in chapter three, as briefly described in appendix-1.

4 .2 .7 Statistical Analysis

Data was summarized as mean ± standard deviation. Statistical difference between means was assessed by paired student t - test and values <0.05 were considered statistically significant.

4. 2 .8 Anion Gap Estimation

The anion gap principle defined as the difference between commonly measured cations and anions $(Na^+ + K^+) - (Cl^- + HCO_3^-)$ (Feddman and Rosenburg,1981) was used to evacuate the level of electrolyte changes. The means were compared using paired t-test and values <0.05 were considered significant.

4.3.0 RESULTS

4. 3 .1 Parasitemia

In the effected group B animals parasites were detected in the infected blood on day 2 p.i, with peak parasitemia occurring on day 5 p.i followed by decline on day 6 p.i and disappearance from circulation on day 7 and 8 p.i, parasites reappeared in circulation on day 9 p.i and was approaching the second peak on day 13 p.i, when the sampling were terminated (Fig 1a). In infected infused Group C animal, parasite appeared on day 2 p.i, peaked on day 5 p.i decreased on day 6 p.i and remained in circulation at very low levels on days 7 to 10 p.i subsequently parasitemia approached a second peak on day 13 p.i (Fig.2b).

4.3.2 Serum Electrolyte Changes

Serum Na^+ levels in infected group B animals was within normal values on days, 3, 4 and 5 p.i, but significantly ($P<0.05$) increased on days 6 and 7 p.i, following decreases and disappearance of parasite from blood. Thereafter, decreases occurred on day 8 p.i producing hyponatremia on day 9 p.i. Subsequently, Na^+ level increased to normal levels on days 11, 12 and 13 p.i, coinciding with the second parasitemic wave (Fig.1a). In the infected and infused group C animals, Na^+ levels were within normal levels on days 3, 4 and 5 p.i, significantly ($p<0.05$) increased on day 6 p.i, but subsequently decreased to remain within normal values during lactose in saline infusion on day 7 to 10 p.i, increases occurred slightly but not above normal values, on day 11, 12 and 13 p.i (Fig.1b). Changes in serum Cl^- expectedly followed the same pattern as serum Na^+ in both groups (Fig. 1a and 1b).

Serum K^+ levels of infected group B animals were within normal range on

day 3, 4, 5 and 6 p.i followed by significant ($p<0.05$) decreases on day 7, 8 and 9 p.i, also coinciding with the decline and disappearance of parasitemia in circulation. Subsequently a gradual increase from day 10 p.i to normal level on days 12 and 13 p.i was observed as the second parasitemic wave built up (Fig. 1a). In the infected group C animals serum K^+ levels were normal on days 3, 4, and 5 p.i, but during lactose infusion on days 6 to 10 p.i serum K^+ levels remained non-significantly lower than those of the uninfected animals. After infusion K^+ levels varied in accordance with the second parasitemic wave (Fig. 1b).

Serum HCO_3^- level was non- significant lower on day; 3, 4 ,5 and 6 p.i but gradually increase on days 7,8,and 9 p.i to within normal values. Subsequent intermittent significantly ($p<0.05$) increase on days 7 to 10 ,11, 12 p.i were seen as the second parasitemic wave up built (Fig .1a). While in the infected and infused group C animals serum HCO_3^- were within normal range on day 3 to 6 p.i, but were significant ($p< 0.05$) lower on days 7 to 10 p.i during lactose in saline infusion. This was followed by significant ($p< 0.05$) increase on days 11,and 13 p.i as the second parasitemic wave built up (Fig.Ib).

All the four electrolyte showed immediate decrease in values upon commencement of lactose in saline infusion on day 6 p.i (Fig. 1b).

4.3.3. Anion Gap Changes

Uninfected control animals showed a gap of 20-22 mMol/l. Infected uninfused animals had an anion gap of 22 mMol/l which significantly increase ($p<0.05$)on day 5 p.i and significantly decrease on days 8 - 10 p.i. While for the infected and infused group C animals, on days 3 to 5 p.i the anion gap significantly

($p<0.05$) increased, but declined the infusion on days (6 to 10 p.i) from the 25 mMol/l to 18 mMol/l and remained within 18 to 22 mMol/l on day 12 to 13 p.i .

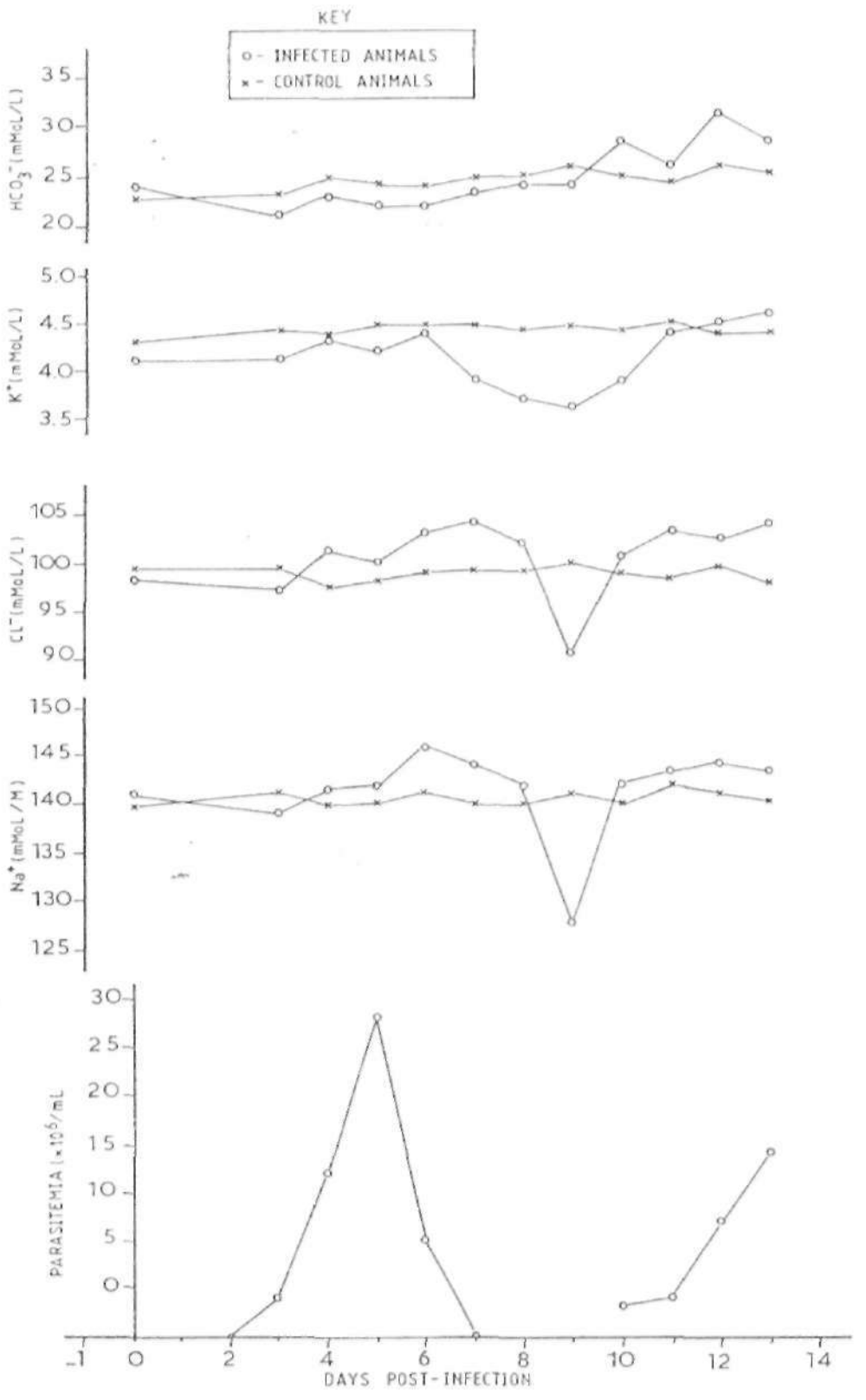


FIG. 1(a): EFFECT OF T. VIVAX INFECTION ON SERUM ELECTROLYTES (Na^+, CL^-, K^+, HCO_3^-) VALUES.

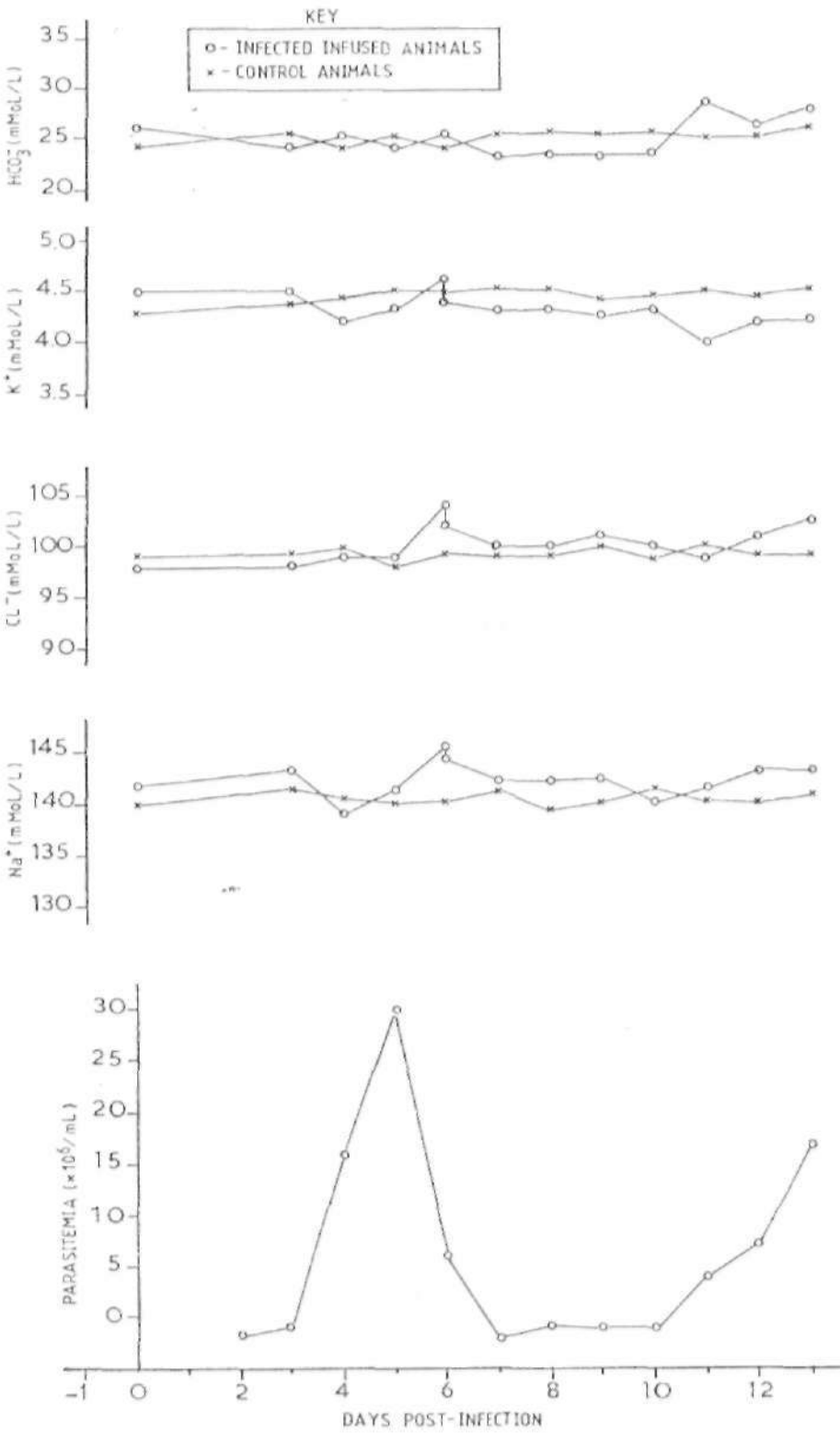


FIG. 1(b) EFFECT OF LACTOSE IN SALINE INFUSION ON SERUM ELECTROLYTES (Na^+, CL^-, K^+, HCO_3^-) LEVELS IN RELATION TO T. VIVAX PARASITEMIA.

4.4.0 DISCUSSION

The infection with *T. vivax* caused an acute disease in the cattle with a very short prepatent period, probably due to the high infective dose of the parasite (Murray and Dexter, 1998). At the onset of the first parasitemia, the four electrolytes of the infected host were maintained within normal range (Fig.1a). The significant ($p< 0.05$) increase in Na^+ and Cl^- following decrease and disappearance of parasites from circulation suggest excretion of these electrolytes into plasma by dying and / or dead trypanosomes. Also, a possible Na-pump modulation might have occurred with increasing parasitemia leading to the inability of the kidney to control the electrolytes. In support of the latter is the observation of a decrease in Na^+- K^+ ATPase activity with increasing parasitemia in *T.congolense* infected rats, leading to modulation of the Na^+ pump (Nok *et. al*, 1992). The observed gradual increases of Na^+, K^+ and Cl^- to slightly above normal values coinciding with the second parasitemia strongly gives credence to causal relationship between level of parasitemia, their metabolic activities directly or indirectly through Na^- pump modulation of the host on the electrolytes and acid-base status of the *T. vivax* infected cattle. Since the excretion, absorption and distribution of Cl^- are passive, usually accompanying Na^+ which is actively transported (Coles, 1986),the similarity in variation of Na^+, Cl^- during this study is explainable.

The decrease in Na^+ and Cl^- on day 8 p.i and the sharp drop on day 9p.i to a point of hyponatremia, and hypochloremia, may have been due to diversion of the excess Na^+ and Cl^- into other body compartments, particularly intracellularly in an attempt to control the excess in circulation, but one would have expected a concomitant increase in plasma K^+ at this period, but it was not so. K^+ level showed

concomitant increase in plasma K^+ at this period, but it was not so. K^+ level showed a significant decrease at this point ,indicating that K^+ is probably being rapidly excreted out.

The increases above normal values of HCO_3^- at the lends seems to further indicate a possible existing metabolic alkalosis due to Cl^- loss and consequently K^+ (Coles, 1986).The kidney in trying to maintain extracellular fluid volume increases Na^+ exchange, H^+ moves extracellular to generate equal amount of HCO_3^-, Hence increase levels of HCO_3^-, as the Na^+ and K^+ exchange increases K^+ may eventually be excreted out with Cl- leading to acid urine frequently observed in metabolic alkalosis (Stephen, 1986: Blood, 1986) However the true metabolic changes would have been better interpreted if the blood PH, carbon dioxide level and hydrogen ion levels were also measured during this experiment.

In group C animals the plasma electrolytes changes during the days of lactose infusion showed normal level of Na^+ and Cl^-, while HCO_3^- and K^+ were below normal levels, this further indicates that the possible existing metabolic alkalosis associated with the disease, may be due partly to K^+ depletion, which did not respond completely to normal saline infusion in the study, as is expected in simple Cl^- loss alkalosis (Stephen, 1985).

Applying the principle of anion gap defined as the difference between commonly measured cations ($Na^+ + K^+$) and anions ($Cl^- + HCO_3^-$) (Fieldman and Rosenberg, 1981) to further evaluate the acid-base status of these animals during the *T. vivax* infection and lactose- in- saline, infusion the anion gap for the uninfected control animals was 20-22 mMol/l. while the anion gap for the infected uninfused group significantly ($p<0.05$) increased on days 4 to 5 p.i as first parasitemia built up,

usually associated with normal or increased anion gap (Maxwell and Kleennan, 1980).

Metabolic acidosis in trypanosomiasis was associated with tissue and organic damage (Fiennes, 1946; Sadun *et. al.*, 1973; Murray *et. al.*, 1974; Ogunsanmi *et. al.*, 1992). Therefore one may surmise that possible metabolic acidosis early in this experiment, as indicated by the anion gap changes, when no severe organic damages may have occurred, might have resulted from a combination of these factor; increase in parasitemia, accompanied by decrease in pcv, hence decreased amount of oxygen carrying capacity of red blood cells to tissues causing sever hypoxia, which enhanced glycolysis and liberation of lactic acid (Fieldman and Rosenberg, 1981). This is because increase anion gap has been associated with increase concentrations of unmeasured organic acids (lactic acid and ketones) and inorganic acids (sulphates and phosphates) (Emmett and Madias *et. al.*, 1979).It is also possible that some degree of tissue damage might have taken place early in the disease. Decline in the anion gap to significantly low levels on days 7, 8 and 9 p.i due to decreased caution values further support the suggestion earlier of existing metabolic alkalosis at this period and metabolic alkalosis in cattle due to the deficient cations placing limits to the kidneys to generate the acid-base balance has been observed (Blood, 1986).

In the infected infused group C animals the anions gap increased significantly ($p<0.05$) prior to infusion from days 3 to 5 p.i similar to infected uninfused at this period, but lactose in saline infusion caused a drop from 25mMol/l on day 7 p.i, a variation close to the uninfected uninfused control animals,

However the gap remained within normal values as the second parasitemia built up.Therefore one may to surmise that in acute *T. vivax* infection of cattle there

seems to be metabolic acidosis, first 5 days of acute infection followed by possible metabolic alkalosis due to depletion of CL^- and K^+. The choice of saline therefore as a solvent for lactose and indeed the total infused volume had no detrimental effects on the hosts electrolytes and acid - base status. Rather the infusion ameliorated the electrolytes abbreviation associated with *T. vivax* infected in cattle; probably by expanding extracellular fluid volume with chloride (Adroque *et. al.* 1978; Madias *et. al.* 1979), therefore the near normal anion gap observed during the infusion. However the addition of Potassium into saline solution will be more beneficial iif lactose-in-salineis to be considered as a base for any trypanocide. (Esievo *et. al.* 1994; Ibrahim *et. al.* 1997; Umar *et. al.*, 1997a; 1997).

CHAPTER FIVE

5.0.0 EFFECT OF LACTOSE IN SALINE INFUSION ON SERUM BIOCHEMICAL PARAMETERS OF CATTLE INFECTED WITH *TRYPANOSOMA VIVAX*

5: 1: 0 INTRODUCTION

Trypanosoma infections have been reported to elicit several changes in the host metabolic system (Seed *et. al.* 1985) and these changes have noticed to be associated with the level of parasitemia of the early phase of the disease (Anosa, 1988) and organ /tissue damage (Fiennes, 1946; Murray *et. al.* 1974; Nok *et. al.*, 1992; Ogusanmi *et. al.* 1994).

Recently histopathological changes were reported in the liver, kidney, spleen and other related organs of cattle infected with *T. vivax* and improvement of these organs were observed following the infusion of lactose in saline into the infected cattle (Ibrahim *et.el.*, 1997). This aspect of the study was therefore designed to observe the variations in the serum biochemical parameters, associated with organ and tissue damage caused by *T. vivax* infection in cattle, and the effect of lactose in saline infection on these parameters

5.2.0. MATERIALS AND METHODS

5.2.1 Experimental Animals

Twelve (12) zebu calves were used and were given the same treatment as those in. 4:2:1

5 .2 .2 Parasite and Infection

Same strain of *Trypanosoma vivax* earlier in 4: 2: 2 was used for the infection and each group of animals was treated as described in 4: 2: 2

5.2.3 Lactose Preparation/Infusion

Same preparation and method of infusion was used as earlier described in 4:2:3

5.2.4 Sample Collection

Same as described in 4:2:4

5.2.5 Estimation of Parasitemia

As in 4:2:5

5.2.6 Sample Analysis

Serum biochemical parameters (Glucose, Billrubin, BUN, albumin and AST) were determined by the methods earlier described in chapter 3 (3: 2: 4).

5.2.7 Statistical Analysis

The student t - test was used and values of $p<0.05$ were considered statistically Significant.

5.2.8 Anion Gap Estimation.

As described in 4: 2: 8

5.3.0 RESULTS

5.3.1 Parasitemia

The parasetimia followed the same pattern as described in chapter 4 (4:3:1), for the infected uninfused group B animals. Similarly for the infected infused group of animals (see Fig. 2a & b, 3a & b, 4a & b, 5a & b & 6a & b).

5.3.2 Packed Cell Volume (Pcv).

The mean pcv for all the 3 groups of animals used for this study, shows that the fluctuation in pcv of normal healthy uninfected calves were within normal range. The drop in mean pcv in *T. vivax* infected calves coincide with the declined in the first peak parasitemia, while decline in mean pcv appeared to have started earlier in the lactose in saline infused group of calves (see Table 2).

5:3:3 Serum Glucose

Serum glucose levels were significantly ($p<0.05$) low on days 3, 4 and 5 post infection (p.i) corresponding with first parasitemia built up. This was followed by significant ($p<0.05$) increases on days 7 and 8 p.i following dissapearance of parasites from blood circulation . Subsequently a gradual decrease from day 10 p.i to below normal values was observed on days 11,12 and 13 p.i as the parasitemia built up (Fig. 2a).

In Group C animals, serum Glucose levels were significantly ($p<0.05$) below normal levels on days 3,4 and 5 p.i as parasitemia built up, but increase to within normal levels on 6 p.i when parasites have significant decline from circulation. During lactose in saline infusion on days 7,8,9 and 10 p.i serum glucose levels were

within normal values of the control group, subsequently , a gradual decrease on days 11,12 and 13 p.i below normal values was observed corresponding with second parasitemia built up. (Fig. 2b)

5:3:4 Serum Bilirubin

Serum bilirubin values for infected uninfused animals were within normal values on days 3,4,5 and 6 p.i as the first parasitemia built up. This was followed by significant ($p<0.05$) increases on days 7,9,11 and 13 p.i as parasites disappear and second parasitemia wave built up (Fig. 3a).

In group C animals serum bilirubin was below normal on day 3 p.i, significantly high on day 4 p.i and was normal levels on days 5 and 6 p.i. During lactose in saline infusion on days 7,8,9 and 10 p.i serum bilirubin values remain with normal values. Subsequently significant ($p<0.05$) increases was noticed on days 11,12 and 13 p.i, as the second parasitemia built up (Fig. 3b).

5:3:5 Serum Albumin

Serum albumin values of infected uninfused animals was low on day 3 p.i and increased to within normal range on days 4,5 and 6 p.i as the first parasitemia built up; followed by significant decreases on days 7,8 and 9 p.i as parasites disappears in circulation, subsequently a gradual increase was observe on days 10 and 13 p.i corresponding with second parasitemia built up (Fig. 4a).

In group C animals serum albumin levels was low on day 3 p.i but increased to normal levels on days 4,5 and 6 p.i corresponding with first parasitemic built up. Subsequently albumin values decreased to significant ($p<0.05$) low levels on days

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7,8,9 and 10 p.i during lactose in saline infusion. This was followed by a gradual increase to above normal value on day 13 p.i as second parasitemia wave peaked (Fig. 4b).

5:3:6 Serum Urea Nitrogen (Bun)

Serum BUN of infected uninfused animals was significantly ($p<0.05$) low on day 3 p.i, but increase to within normal levels on day 4, 5 and 6 pi as first parasitemia built up, this was followed by significant ($p<0.05$) decreases on days 7, 8 and 9 p.i as parasite disappeared in blood circulation. A non-significant increase on days 10, 11, 12 and 13 p.i was observed as second parasitemia built up. (Fig. 5a)

In group C animals, serum BUN pattern of change was similar to that of group B on days 3 to 5p.i, but significantly ($p<0.05$) increased on days 6 and 7p,i during lactose infusion. Subsequently significant ($p<0.05$) decreases were observed on days 8 to 13p.i when the experiment was terminated.(see Fig.5a).

5.3.7 Serum Aspartate Aminotransferase:(AST)

Serum AST was low on days 3 and 4 p.i and increased with increased parasitemia to within normal value on days 5,6,7 and 8 p.i. A significant ($p<0.05$) decrease was observed on day 9 p.i and a gradual increase was observed as second parasitemia built up (Fig. 6a).

In the group C animals serum AST was low on days 3 and 4 p.i and increased to peak as parasitemia peaked; this was followed by significant ($p<0.05$) decreases on days lactose in saline was infused and post infusion. (Fig. 6b).

TABLE- 2: THE MEAN PERCENTAGE PACKED CELL VOLUMES (PCV) OF UNINFECTED ZEBU CALVES; *T. VIVAX* INFECTED CALVES AND *T. VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES COMPARED TO THE PARASITEMIA.

DAYS POST INFECTION (P.I)	MEAN %PCV OF UNINFECTED ZEBU CALVES (MEAN ± SEM)	MEAN % PCV OF *T.VIVAX* INFECTED ZEBU CALVES (MEAN ± SEM)	PARASITEMIA OF INFECTED ZEBU CALVES ($X10^6$/ML)BLOOD	MEAN % PCV *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (MEAN ± SEM)	PARASITEMIA OF INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES ($X0^6$/ML) BLOOD.
0	24.67±1.67	21.33±0.67	0	25.00±0.82	0
2**	24.33±1.86	20.33±0.34	0	24.75±1.25	+3
3**	24.33±1.33	19.67±0.34	+4	23.75±1.11	+4
4	24.33±1.33	19.67±0.34	11.50	25.00±0.82	15.50
5	24.33±1.33	19.00±1.00	28.00	23.00±1.00	29.00
6	24.67±1.67	17.00±1.00	5.40	20.80±1.04	5.50
7**	24.33±1.86	16.00±1.53	0	20.75±1.75	+3
8**	24.33±1.33	13.33±1.86	0	18.75±1.03	+4
9**	24.33±1.86	15.67±1.67	0	20.00±0.92	+4
10**	24.33±1.86	15.33±1.46	+3	19.00±0.57	+4
11**	25.33±1.46	15.67±1.20	+4	18.00±0.92	3.50
12	25.33±1.46	19.33±0.34	6.50	18.25±0.62	6.50
13	24.33±1.33	15.67±0.67	10.50	16.50±0.29	16.00

** Parasitemia of these days were too low to be counted by the haecytometer. They were estimated by microhaematocrite method as
1+ = an occasional parasite per field
2+ = 1 to 5 parasitesd per buffy coat area
3+ = 5 to 10 parasites per buffy coat area
4+ = 10 to 20 parasites per buffy coat area

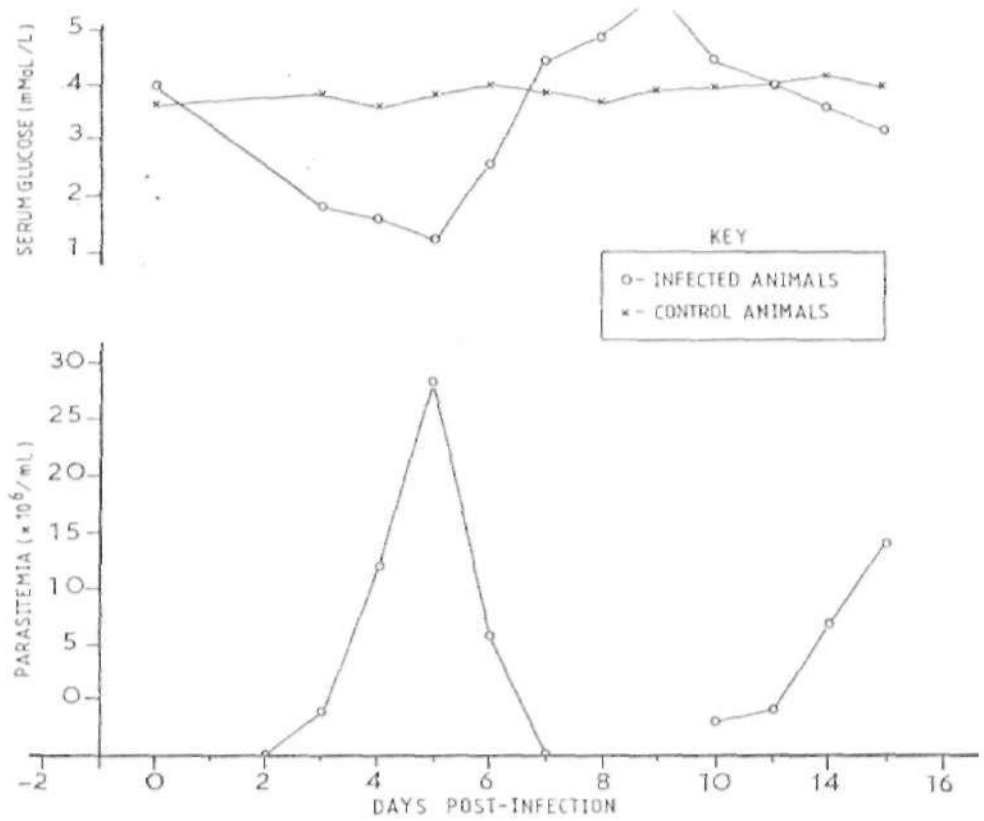


FIG. 2(a). EFFECT OF T. VIVAX INFECTION ON SERUM GLUCOSE LEVELS IN RELATION TO PARASITEMIA.

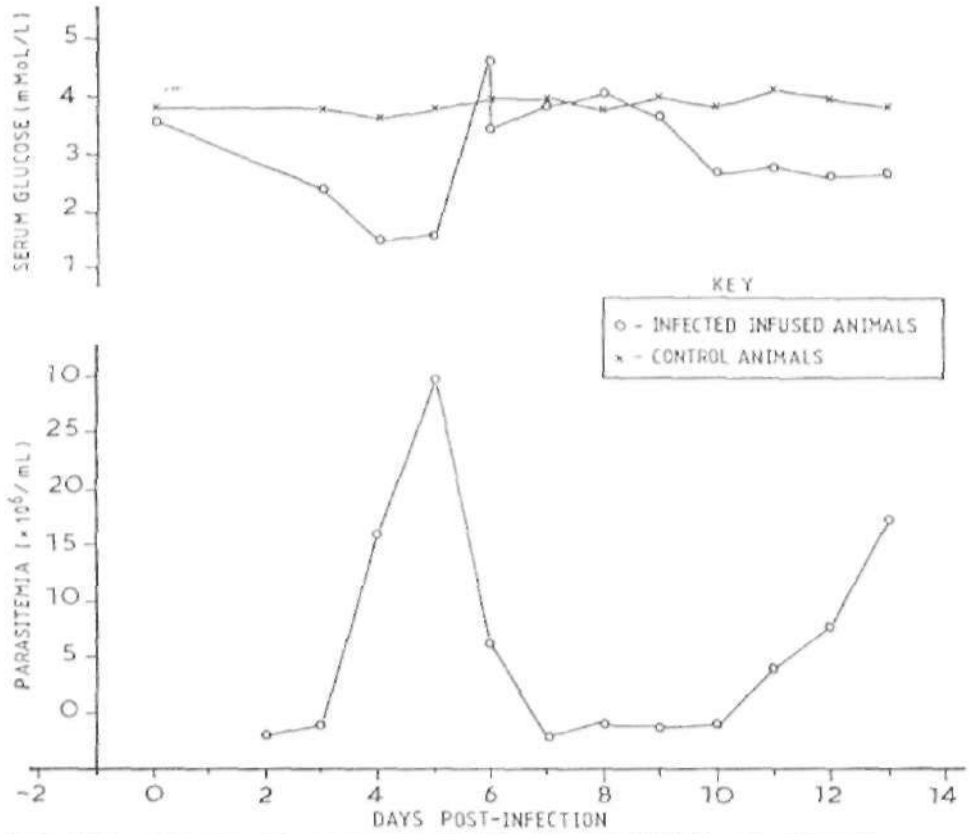


FIG. 2(b). EFFECT OF LACTOSE-IN-SALINE INFUSION ON SERUM GLUCOSE IN RELATION TO T. VIVAX PARASITEMIA.

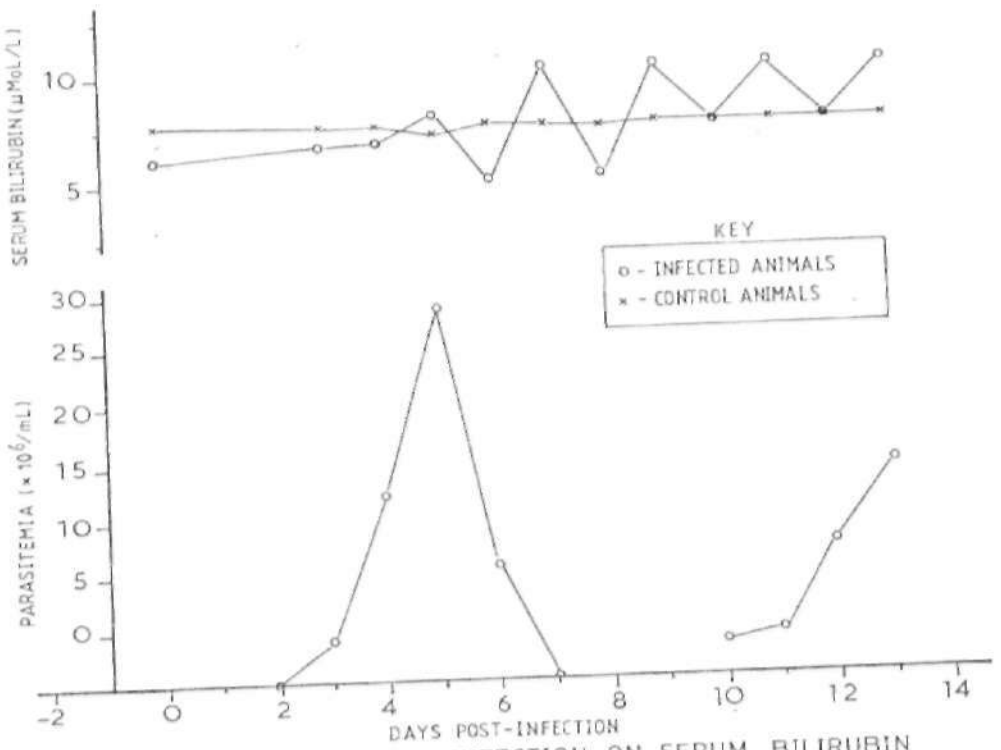


FIG. 3(a). EFFECT OF T. VIVAX INFECTION ON SERUM BILIRUBIN LEVELS IN RELATION TO THE PARASITEMIA.

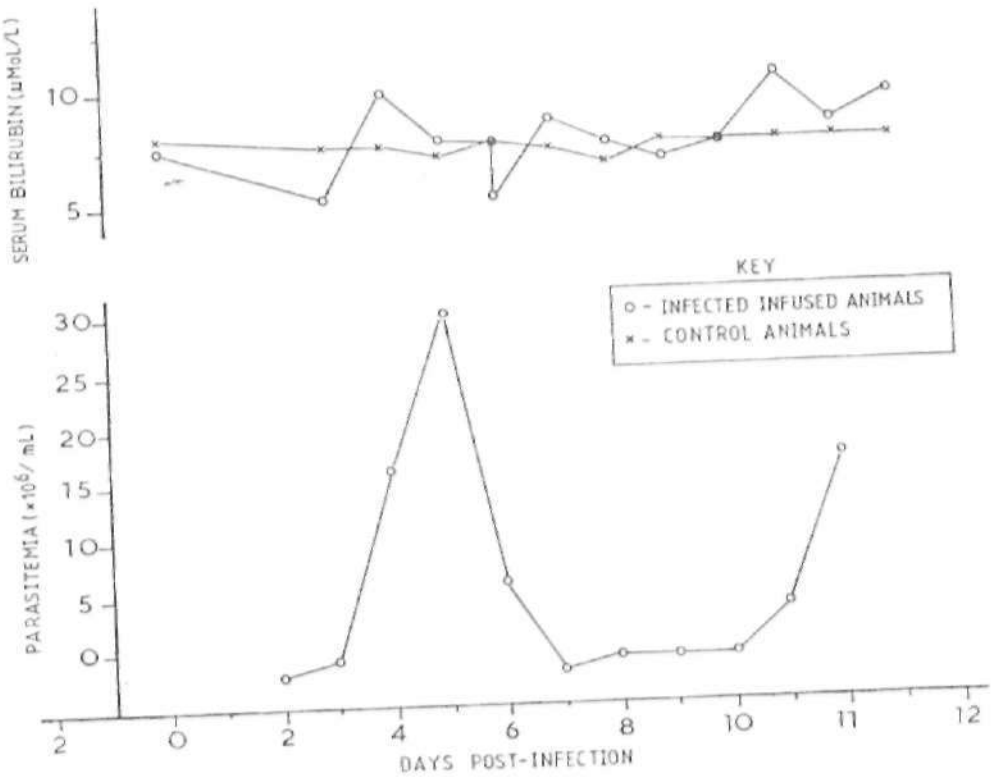


FIG. 3(b). EFFECT OF LACTOSE-IN-SALINE INFUSION ON SERUM BILIRUBIN IN RELATION TO T. VIVAX PARASITEMIA.

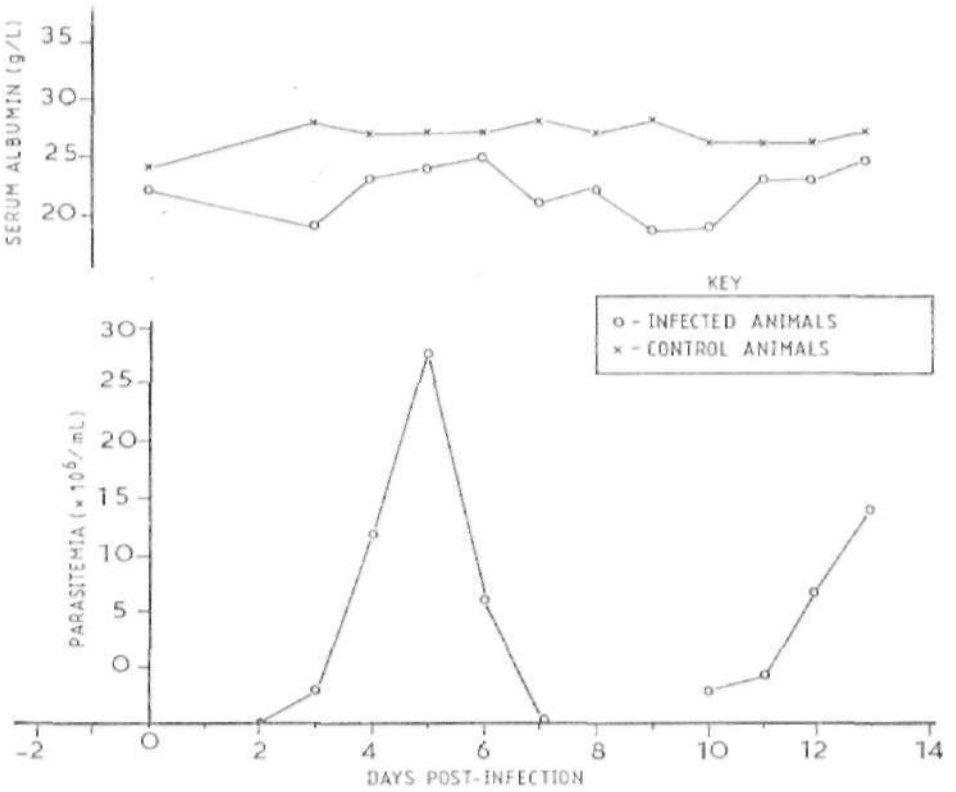


FIG. 4(a). EFFECT OF T. VIVAX INFECTION ON SERUM ALBUMIN LEVELS IN RELATION TO THE PARASITEMIA.

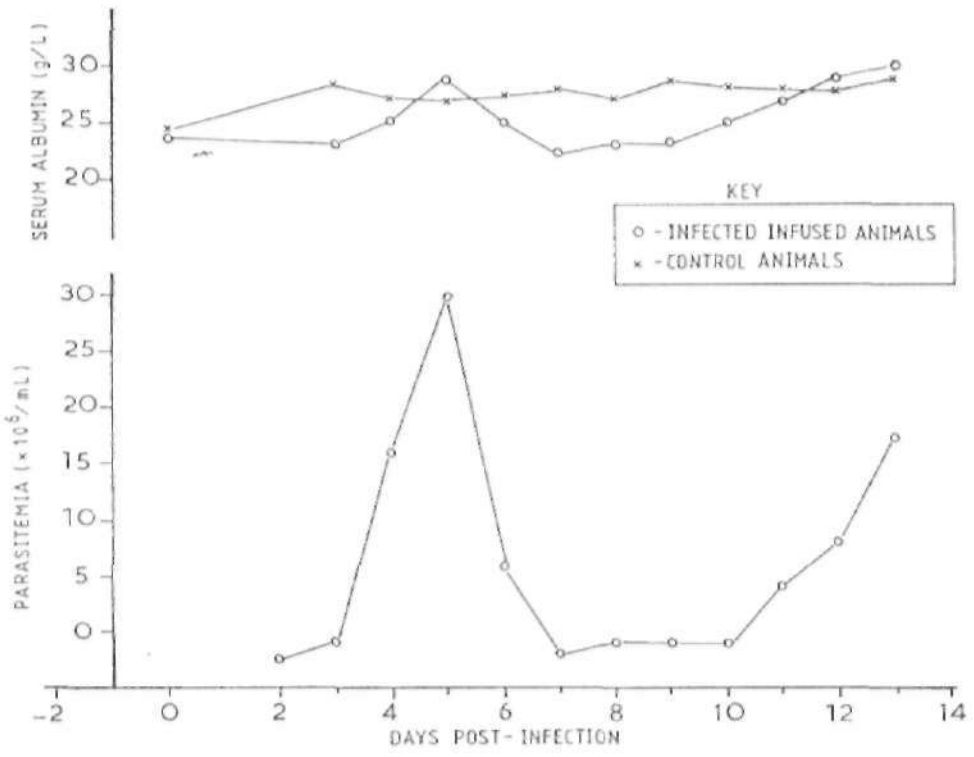


FIG. 4(b). EFFECT ON LACTOSE-IN-SALINE INFUSION ON SERUM ALBUMIN LEVELS IN RELATION TO T. VIVAX PARASITEMIA.

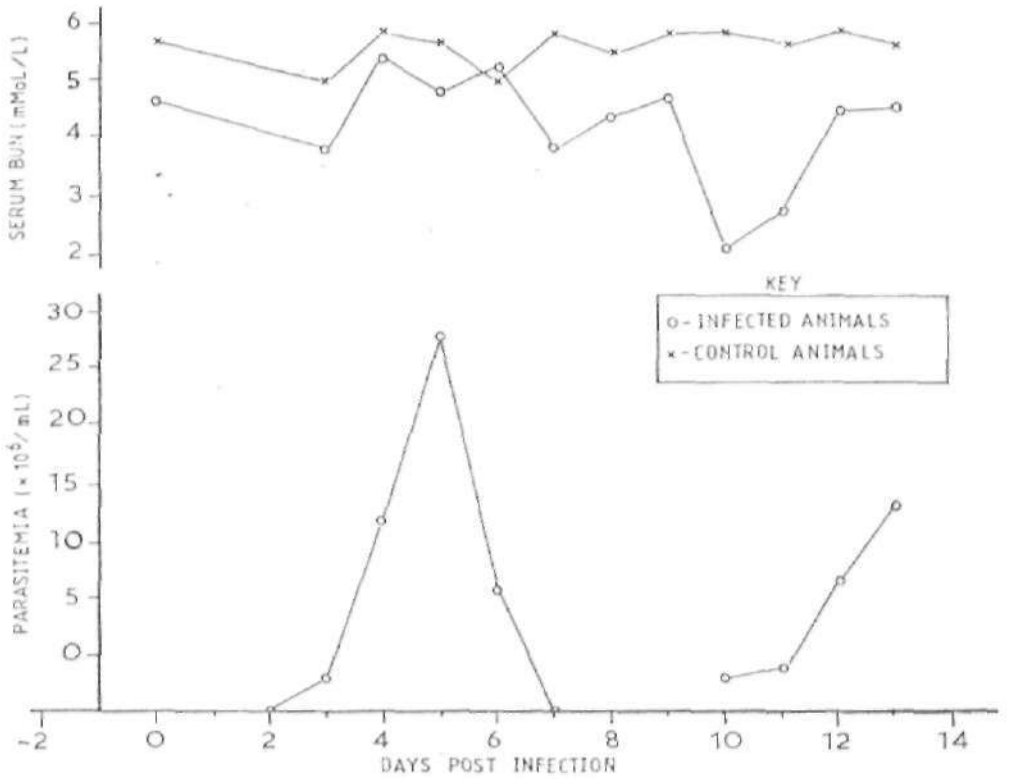


FIG. 5(a). EFFECT OF T. VIVAX INFECTION ON SERUM BUN LEVELS IN RELATION TO THE PARASITEMIA.

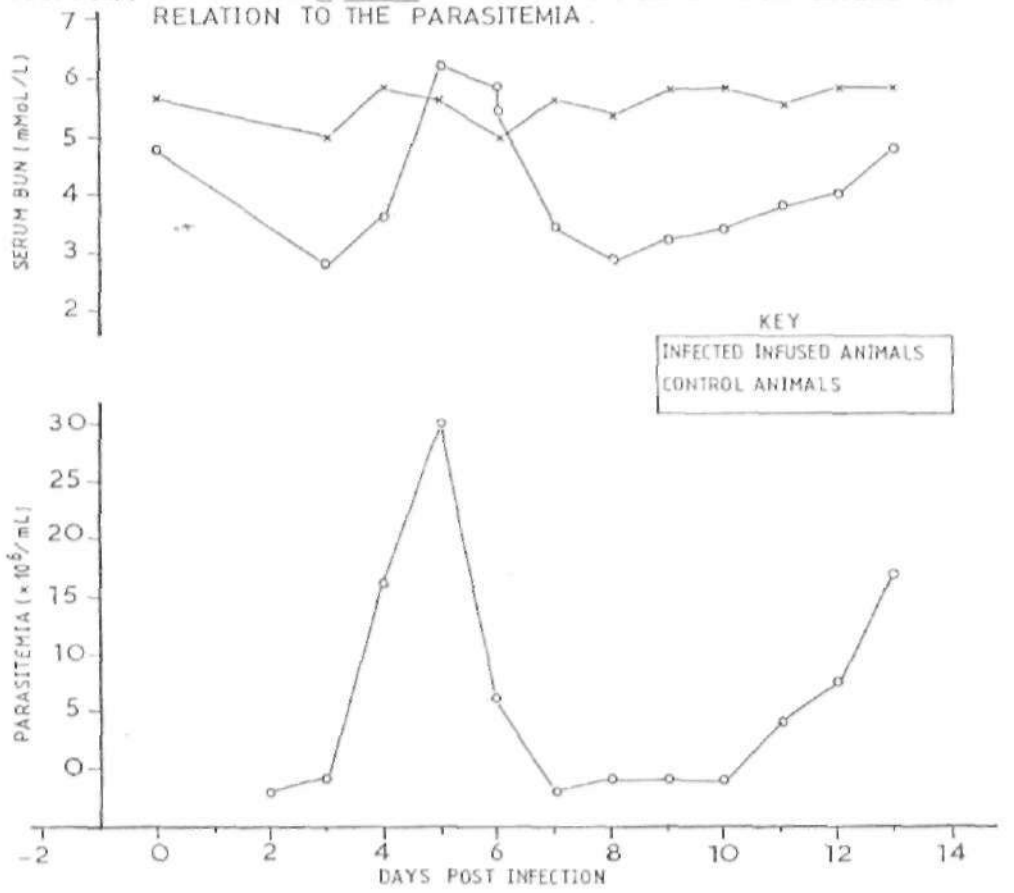


FIG. 5(b). EFFECT ON LACTOSE-IN-SALINE INFUSION ON SERUM BUN IN RELATION TO T. VIVAX PARASITEMIA.

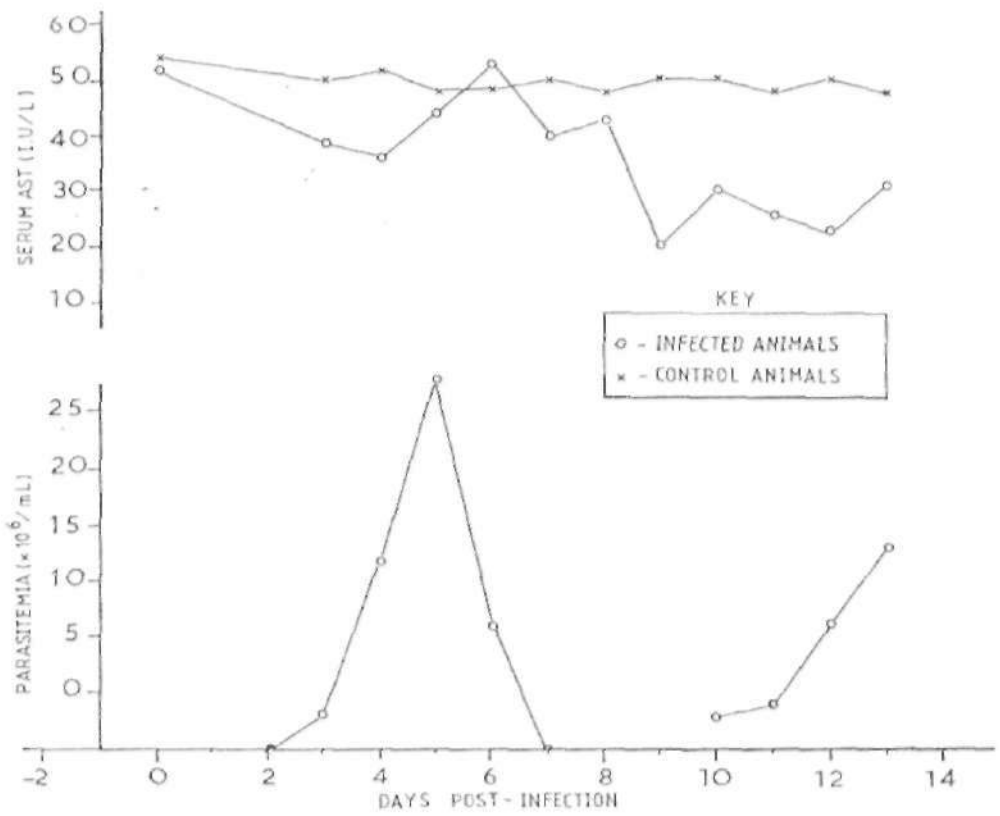


FIG. 6(a). EFFECT OF T. VIVAX INFECTION OF SERUM AST LEVELS IN RELATION TO PARASITEMIA.

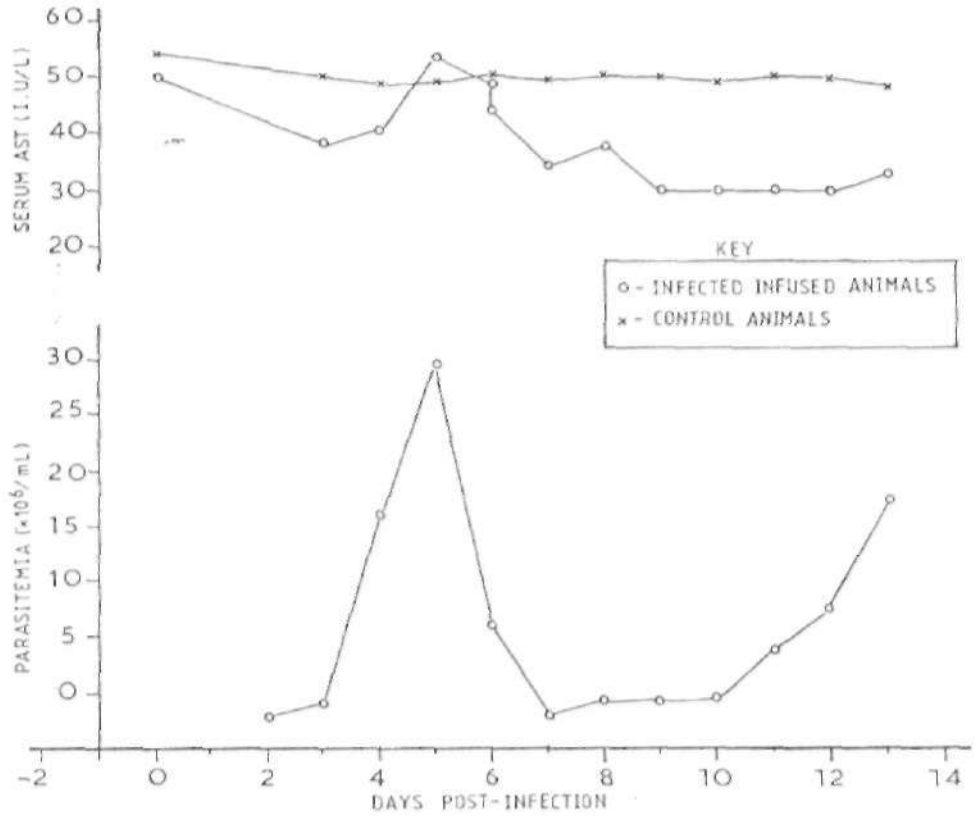


FIG. 6(b). EFFECT OF LACTOSE-IN-SALINE INFUSION ON SERUM AST IN RELATION TO T. VIVAX PARASITEMIA.

5: 4: 0. DISCUSSION

Serum glucose levels were significantly low on days 3, 4, 5 and 6 p.i as parasitemia built up. This agreed with Hudson (1944), Moon *et. al.* (1973), Singh and Guar (1983), Jani *et. al* (1992) and Joshua *et. al.* (1985), that serum glucose levels decrease at periods of high parasitemia in acute trypanasomiasis, presumably the large number of parasite deplete host carbohydrate reserve and utilizes the glucose for their glycolytic pathway (Opperdoes & Borst, 1977.)

The significant increases observed as parasites disappear from circulation on days, 7 and 8 p.i, indicates glucose accumulation; may be due to normal glucose output by the liver, but decreased peripheral removal (Coles, 1986) as a result of decreased Na^+ which is necessary in the Na^+- glucose symport transport (Ramazan *et. al.* 1996). Therefore, decreased Na^+ may have reduced tissue ability to utilize glucose, hence the high level in plasma at that period.

In the infected infused group, serum glucose levels, were significantly ($p<0.05$) low as parasitemia built up, but was within normal levels during lactose in saline infusion days. However, with parasite in circulation during the infusion one expected lower levels of glucose than observed, but it was not so, agreeing with suggestion that lactose may have been hydrolysed to galactose and glucose, thereby providing the parasites in circulation ready glucose for metabolism (Umar, 1997). Although it is also possible that the saline solution might have been supplying readily available Na^+ for effective Na^+ - glucose symport to transport glucose to peripheral tissues for use, since normal plasma Na^+ at the period (Fig. 1b) was observed. The further decrease in glucose levels on days 11, 12 and 13 p.i following second

parasitemia give more credence to the casual relationship between parasitemia and depletion of host plasma glucose.

The non-significant changes in the serum values of bilirubin on days 3, 4, 5 and 6 p.i following parasite built up, and in presence of decreasing pcv, suggest that although there may be red blood cells destruction taking place, there may also be an enhancement of the liver functional reserve to conjugate the excess bilirubin produced (Coles, 1986). While the significant ($p<0.05$) increases on days 9 to 13 p.i coincides with the second parasitemia built up, and agrees with Fiennes (1970; 1954), Hudson (1944), Naylor (1971) and Stephen and Mackenzie (1959) that in *T.congolense* infections in cattle, elevated serum is observed in acute infection and terminally in chronic cases.

In the infected infused group, the significantly ($p<0.05$) high level of serum bilirubin day 4 p.i might have been due to the large number of parasites, hence increased cell destruction, and increased bilirubin for liver conjugation, while the normal values on days, 5 and 6 p.i may be indicating increased liver ability to conjugate the bilirubin. During lactose infusion however on days 6 to 10 p.i serum bilirubin values remained within normal range, this could be due to a combination of factors, either the lactose in saline caused an increased plasma volume, hence dilution of the high levels, or the lactose infusion may have reduced the process of erythrophagocytosis and subsequently reduction in bilirubin production ,thus the normal value observed. The subsequent increases on days 11, 12 and 13 p.i as the second parasitemia built up, further indicates that increased bilirubin values relates to the parasitemia in this infection.

The increased value of BUN observed agrees with the findings of Hudson

(1944), and Isoun *et. al* (1979) that cattle infected with *T.vivax* have increased BUN at periods of high parasitemia in acute infections, probably due to the pyrexia resulting in catabolic breakdown of body proteins leading to the increases in plasma levels, while the decrease on days 7 and 8 p.i coinciding with absence of parasites may be due to non catabolic breakdown of proteins in the absence of the parasites, or a combination of an accelerated excretion of BUN by the kidneys, the increases later as the second parasitemia built up, may arise from catabolic breakdown of proteins. However during lactose in saline infusion the significant ($p<0.05$) decreases could have been due to haemodilution, while the serum increases on day 11, 12 and 13 p.i second parasitemic wave built up could have been due to the level of parasitemia.

Serum albumin levels was significantly ($p<0.05$) low on day 3 p.i., but rose to within high levels even though still below normal values on days 4, 5, 6 and 7 p.i, corresponding with first parasitemia built up. This apparent increase as parasite build up, agrees with Tizard and Holmes (1978) that in the course of acute trypanosomiasis the level of albumin increase primarily to detoxify excess fatty acid released from red blood cell membranes due to the activity of the parasites, the decreases on days 8 and 9 p.i further supports this observation, while the increase on days 11, 12 and 13 p.i as second parasitemia built up, further gives credence to the relationship between parasitemia and albumin changes (Fig. 4a). The low levels of albumin observed throughout the experiment, indicates that the animals used for the infection had lower protein levels than those of the control animals. The serum albumin changes in the infected infused group, followed a similar pattern as the uninfused group from days 3, 4, 5 and 6 p.i, while during lactose in saline infusion, the serum albumin

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levels were significantly ($p<0.05$) low on days 7 to 10 p.i the decrease may be due to plasma volume solute dilution including the albumins (Coles, 1986). While the subsequent increases to above normal values on days 11, 12 and 13 p.i as the second parasitemic wave built up, agrees with Tizard and Holmes (1978), and Anosa (1988) who attributed the varrying serum protein level to the degree of parasitemia.

Serum aspartate aminotransferase (AST) levels increases with parasite built up on day 5 and 6 p.i and remained within normal levels on days 7 and 8 p.i as parasite disappears in blood. This increases early in the disease could have been due to a combination of factors, such as parasites releasing AST as part of their metabolites, since high levels of AST and alanine aminotransferase (ALAT) have been observed in homogenates and suspensions of living trypanosomes (Stephen and Gray, 1960, Gray 1961) and a possible organ damage may have occurred at this same period due to the parasites presence.

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CONCLUSION

The infection of cattle with the Samaru strain of *T.vivax* in this study caused a variety of changes in the electrolytes and biochemical parameters, and consequently the acid-base status of the animals under study.

The possible early acidosis occurring the first 5 days p.i was probably due to a combination of factors: a decreased pcv as parasitemia built up, and consequently hypoxia leading to lactic acid accumulation in tissues. And/or a probable low level of tissue damage early in the disease. The metabolic alkalosis observed immediately following acidosis as parasites decreased and disappeared from circulation, further indicates, possible decrease in Na^+ - K^+ Atpaseactivity with incresing parasitemia and consequently Na-pump modulation leading to kidney inability to regulate electrolytes during the infection.

Lactose in saline solution was infused at the peak of parasitemia, the infusion and the volume given appeared useful in correcting the acid-base changes observed earlier, however the lack of HCO_3^- and K^+ returning to normal values during the infusion further gives credence to the assumption that the possible alkalosis observed may have been due to K^+ depletion since in simple Cl^- loss alkalosis, normal saline therapy should correct the alkalosis. Therefore if the infusion of lactose in normal saline is to be significant from the viewpoint of chemotherapy of bovine trypanosomosis, the low levels of K^+ has to be accertain if it is due to actual K^+ depletion, then K^+ containing fluid should be infused during the infection.

The serum glucose changes observed seems to indicate that Na^+ -glucose symport transport activity into cells is affected by *T.vivax* infection, but the lactose-saline infusion serves to correct this malfunction.

The changes in serum bilirubin, albumin, urea and AST during the lactose in saline infusion is attributed to factors, such as increase plasma volume leading to proteins dilution, and also to the fact that the infusion may have served to reduce the level of tissue damage that might have occurred due to the infection, hence the variations in these parameters.

Therefore the use of lactose in saline and the quantity infused appear to be useful incorrecting the electrolytes changes and seems not to cause any serious tissue reaction, judging from the changes observed in the blood biochemical parameters, probably by expanding the extracellular fluid volume with Cl^-, and allowing H^+ excretion, therefore the near normal anion gap observed. However the addition of K^+ ions into the lactose in saline solution may be more beneficial if lactose in saline is going to be consider as a base for a trypanocide.

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APPENDIX-I

SUMMARY OF METHODS USED IN THE ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS

1. NA^+ AND K^+ DETERMINATION BY FLAME EMISSION SPECTROMETRY (CHEESEBROUGH, 1991)

Principle:

Using compressed air, diluted serum is sprayed as a fine mist of droplets into a non-luminous gas flame. These then gives the colour of emission characteristics of Na^+ or K^+ metallic ions.

A light filter select the light of a wavelenght corresponding to the metal being measured. This is then allowed to fall on a photosensitive detector system which then emits lights, the amount of which depends on the concentration of metallic ions. The value is the evaluated in mMol/l.

2. SERUM BICARBONATE ESTIMATION BY TITRIMETRIC METHOD (CHEESBROUGH, 1991)

Principle:

A known amount of strong HCl is added to sample of serum, the reaction forms $C0_2$ which is expelled by shaking. The H^+ remaining are titrated against NaOH with phenol red as indicator.

Method:

Preparation of comparison solution.

Pipette into conical flask or beaker

*NaCl (10g/1)-	6.0ml
*Serum sample (treated with antifoaming agent)-	0.1ml
*Phenol red indicator -	0.1ml

Mix well.

$HC0_3$. measurement

The following are pipetted into conical flask or beakers.

	Standard		Test.	
	S1	S2	T1	T2
NaCl (10g/1)	4 ml	4 ml	4 ml	4 ml
HCO_3- Standard 25mMol/1	0.1ml	0.1ml	-	-
Serum sample (treated with antifoaming agent)	-	-	1 ml	1 ml
HC1 mMol/1	1 ml	1 ml	1 ml	1 ml

The content of each flask is then shaken to expel $C0_2$, then allow to stand for 1 minute, 0.1 ml phenol red indicator is then added to each flask, then titrated with Na0H 10mMol/1 unit the colour matches the comparison flask.

Concentration of $HC0_3$- =A x 100 mMo1/1
Where A = 1.00 - volume of Na0H used
Then the mean of duplicate tests estimated = 1 mMo1/1

3. SERUM TOTAL BILIRUBIN ESTIMATION BY MODIFIED METHOD OF JENDRASSIK AND GROF (1958) AS OUTLINED BY CHEESEBOUGH (1991)

Principle:

Reaction between sodium nitrate and Hydrochloric acid produces nitrous acid, which is then used to diazolised sulphuric acid. This is then used to react with the bilirubin in the serum sample to form a pink azobilirubin acid. This is later converted to a blue coloured azobilirubin acid with alkaline tartrate reagent then the absorbance of the blue-green solution is red in a colorimeter with an orange filter or in a spectrophotometer at wavelength 600nm.

Summary

Titration into Tubes	Standard S.	Sblank	Control C.	Cblank	Tests 1.2 etc	1.2etc
1.Caffeine Benzoate Reagent	lml	lml	lml	lml	lml	l m l
2.Standard serum	0.lml	0.lml	-	-	-	-
3.Control serum	-	-	0.lml	0.lml	-	-
4.Serum sample	-	-	-	-	0.lml	0.lml
5.Diazo reagent	0.5ml	-	0.5ml	-	0.5ml	-
6.Sulphuric acid 5g/1	-	0.5ml	-	0.5ml	-	0.5ml

These are thoroughly mixed, then left at room temperature for 5 minutes. Alkaline tartrate reagent is then added and mixed well.

Alkaline tartrate	lml	lml	lml	lml	lml	lml

Then the absorbances are then read using spectrophotometer at 600nm (Colorimeter with orange filter) by initially zeroing the instrument with distilled water, the blank are first read and their reading substracted.

Total bilirubin μmo1/1 = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ X Concentration of standard

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4.ESTIMATION OF SERUM ALBUMIN AS OUTLINED BY CHEESEBROUGH, (1991)

Principle:

Bromocresol green (BCG) albumin method is used. BCG is an indicator. It is yellow between pH3.5 - 4.2. on binding to albumin the colour changes from yellow to blue green.

Albumin + BCG pH 4.2 -- > Albumin BCG complex (Blue Green). The absorbance of the colour produced is measured in a colorimeter via an orange filter or in a spectrophotometer at 632nm wavelength. To eliminate turbidity, a reagent Brij -32 is added.

Summary

Pipette into tubes	Blank B	Standard S	Control C	Test 1,2, etc
BCG reagent	4 ml	4ml	4 ml	4 ml
Distilled water	20μl	-	-	-
Standard 30g/1	-	20μl	-	-
Control serum	-	-	20μl	-
Serum sample	-	-	-	20μl

The test tubes are then shaken to mix well (in such a way as to aviod frothing). Absorbance is read immediately using either colorimeter (orange filter) or spectrophotometer at 632nm. The instrument is zeroed with blank solution intube B.

$$\text{Albumin g/1} = \frac{\text{Absorbance of test control}}{\text{Absorbance of 30g/1 Standard}} \times 30$$

5. BLOOD OR SERUM UREA ESTIMATION BY METHOD OF STROVE AND MAKOROVA, (1989)

Principle:

Urea forms a red coloured compound when reacted with diaceryl monoxime in the presence of thiosemicarbazide and Iron Salts in a strongly acid medium, with the intensity of the red colouration being proportional to the concentration of urea of the analysed sample.

Summary -

A proportion of the serum is diluted 10 times

Pipette into tubes	Standard	Control	Test
Serum sample (portion diluted 10 times	-	-	0.1.ml
Standard Urea solution	0.1.ml	-	-
Distilled water	-	0.2.ml	-
Colour reagent	2.ml	4.ml	2.ml

Mix contents well with shaking. Cover the tubes with aluminium foil and place them in a boiling water bath for about 10 minutes. Remove and cool under stream of running water. Then measure the absorbance of the standard and sample solutions against the control solution on a photocolorimeter with green light filter at 540 - 560nm.

$$\text{Blood or Serum Urea concentration mMol/l} = \frac{\text{E sample } 16.65}{\text{E standard}}$$

E sample = Absorbance of sample solution measured against control solution
E standard = Absorbance of standard solution measured against control solution
16.65 = Concentration of Urea in Standard Solution in mmol/l

$$\text{The Urea nitrogen in mmol/l} = \frac{\text{Blood Serum Urea Concentration}}{214}$$

$$214 = \frac{\text{Urea Molecular Mass (30)}}{\text{Urea nitrogen molecular mass (28)}}$$

6. SERUM ENZYME (ASPARTATEAMINOTRANSFERASE) ESTIMATION USING THE MODIFIED REITMAN AND FRANKEL METHOD AS OUTLINE BY CHEESEBOUGH, (1991)

Principle:

AST is incubated at 37°C for 1 hour in a pH 7.5 buffered substrate containing aspartate and a-ketoglutarate. AST catalyses the transfer of the amino group from aspartate to ketoglutarate to form oxaloacetate and glutamate. The former then reacts with 2,4-dinitrophenyl hydrazone, which in an alkaline medium gives a red-brown colour. The absorbance of this colour is measured using either colorimeter with a green filter or a spectrophotometer at 505nm wavelength.

Aspartate + a-ketoglutamate, pH.7.5 <~ ~> Glutamate + oxaloacetate

37°C, lhr I PNPH AHiBne pi!

Red- brown colour

Summary:

Pipette into tubes	*Blank*	*Standard*	*Control*	*Test*
	B	B	C	1.2etc
Reagent 1 (substrate)	-	-	0.5 ml	0.5 ml

Substrates in tube C and test 1,2 etc are warmed at 37°C for 5 minutes.

Control serum	-	-	0.1 ml	
Serum sample	-	-	-	0.1ml

Incubate tubes C, test 1,2 etc at 37° C for 1 hr. Then just before 1 hr elapse, add

Distilled water	0.1ml	0.1ml		
Reagent (substrate)	0.5 ml	0. 3 ml		

At exactly lhr pipette as follows:

Reagent 3(color reagent)	0. 5 ml	0. 5 ml	0. 5 ml	0.5 ml

Mix well and leave at room temperature for 5 minutes. Then read absorbance using either colorimeter with green filter or spectrophotometer at 505nm. Instrument is zeroed with blank solution in tube value obtained in Ul/F from the calibration graph

7. SERUM GLUCOSE **ESTIMATION USING OXIDASE-PEROXIDE METHOD (CHEESEBROUGH, 1991)**

Principle:

Glucose oxidase (GoD) catalyses the oxidation of glucose to give hydrogen peroxide (H_20_2) and gluconic acid. In the presence of enzyme peroxidase the H_20_2 is broken down and the oxygen released reacts with 4-aminophenozone (4-aminoantipyrine) and phenol to give a pink colour.

The absorbance of the colour produced is measured via a colorimeter using a green filter or a spectrophotometer at 515nm

$$\text{Glucose} + 0_2 \xrightarrow{\text{GoD}} \text{Gluconic acid} + H_20_2$$

$$H_20_2 + \text{aminophenazone} + \text{phenol} \xrightarrow{\text{GoD}} \text{Coloured complex pink}$$

Summary

Pipette into tubes	Blank B	Standard S	Control C	Test 1,2 etc
Protein precipitant Reagent	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Distilled water	50μl	-	-	-
Standard 10(mMol/l)	-	50μl	-	-
Control Serum	-	-	50μl	-
Serum sample	-	-	-	50μl

These samples are then mixed well Tubes C and test 1,2 etc are centrifuged to obtain clear supernant fluids.

Take a second set of tubes and label B,S,C, 12 etc.

Transfer from first to

Second set of tubes	0.5 ml	0.5 ml	0.5 ml	0.5 ml

Add colour reagent to

Second set of tubes	1.5 ml	1.5 ml	1.5 ml	1.5 ml

Mix well incubate at 37^{0}C for 10 minutes, shaking occasionally.

Read absorbance using either colorimeter with green filter or spectrophotometer at 515nm wavelength.

Zero instrument with blank solution in tubes. The resulting values are obtained from calibration graph, if the result of 10 mMol standard agrees with the calibration or Glucose can be obtained by formular.

$$\text{Glucose (mMol/l)} = \frac{\text{Absorbance of test or control}}{\text{Absorbance of 10 mMol/l standard.}}$$

8.SERUM CHLORIDE DETERMINATION BY METHOD OF SCHALES AND SCHALES(1941).

Principle :

The chloride of a biological specimen is titrated in an acid medium with standard mercuric nitrate using 5-diphenylca bazone as an indicator chloride ion reacts with mercuric ion to form the soluble, but non-dissociated mercuric chloride . At the end point when a slight excess of mercuric ion is present a stable violet blue complex is formed with the indicator.

Procedure :

1.Place in a shell vials the following

	Specimen	Standard
H_2O_2	1.0ml	1.0ml
Standard	-	0.1ml
Specimen	0.1ml	-
Indicator	2 drops	2 drops

2.Titrate the contents of each vial with standard mercuric nitrate solution to the first faint trace of violet blue colour.

3.Calculation = $\frac{\text{Xml for unknown}}{\text{Xml for standard}}$ X 100 mMol/L Cl

APPENDIX-2

TABLE A2 - 1: MEAN (M ± SEM) SODIUM (NA^+) VALUES OF UNINFECTED (CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES, AND *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES. (n=4)

DAY POST INFECTION (P.I)	MEAN NA^+ VALUES OF UNINFECTED ZEBU CALVES. (mMOL/L)	MEAN NA^+ VALUES OF *T.VIVAX* INFECTED ZEBU CALVES. (mMOL/L)	MEAN NA^+ VALUES OF *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES. (mMOL/L)
0	140.03 ± 0.92	141.02 ± 1.50	142.00 ± 1.00
3	140.67 ± 0.67	139.57 ± 2.31	138.55 ± 1.80
4	140.07 ± 0.34	142.00 ± 1.16	143.00 ± 0.50
5	140.30 ± 0.34	142.50 ± 2.31	142.00 ± 2.06
6	140.30 ± 1.18	146.00 ± 1.16	145.50 ± 0.75 *144.03 ± 1.08
7	141.03 ± 1.33	144.00 ± 1.16	143.50 ± 1.89 *143.25 ± 1.81
8	140.30 ± 1.46	142.24 ± 2.31	143.50 ± 2.22 *142.50 ± 2.16
9	141.50 ± 0.34	128.00 ± 9.24	142.52 ± 2.62 *142.50 ± 2.59
10	141.30 ± 1.34	143.50 ± 0.89	142.05 ± 2.59 *138.88 ± 2.59
11	140.00 ± 2.00	143.00 ± 1.16	140.50 ± 4.50
12	141.33 ± 1.80	144.04 ± 2.89	143.56 ± 1.50
13	140.33 ± 0.34	144.59 ± 4.80	141.50 ± 5.50

*Post Lactose -in -Saline Infusion values.

TABLE A2-2: MEAN (M ± SEM) SERUM CHLORIDE (Cl^-) VALUES OF UNINFECTED(CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN Cl^- VALUES OF UNINFECTED ZEBU CALVES (mMol/l)	MEAN Cl^- VALUES OF *T. VIVAX* INFECTED CALVES (mMol/L)	MEAN Cl^- VALUES OF *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (mMol/L)
0	98.67 ± 0.39	99.05 ± 3.00	99.50 ± 0.80
3	98.79 ± 0.67	97.60 ± 4.03	99.00 ± 0.58
4	98.17 ± 1.45	101.00 ± 0.57	99.30 ± 1.03
5	98.06 ± 1.53	99.99 ± 1.73	99.0 ± 1.00
6	98.70 ± 0.67	103.00 ± 1.73	103.3 ± 1.97 *102.6 ± 1.80
7	99.30 ± 0.67	104.00 ± 1.16	101.8 ± 2.84 *99.75 ± 1.84
8	98.70 ± 0.88	102.00 ± 0.00	101.0 ± 0.57 *99.75 ± 1.84
9	99.05 ± 0.06	90.00 ± 8.00	102.5 ± 3.40 *98.0 ± 2.95
10	98.30 ± 0.34	101.20 ± 2.89	103.5 ± 2.06 *97.5 ± 2.63
11	98.70 ± 0.67	103.00 ± 0.57	99.00 ± 8.06
12	99.00 ± 0.00	102.00 ± 0.00	101.00 ± 1.29
13	98.38 ± 0.88	104.00 ± 3.46	102.00 ± 5.55

* Post lactose-in-saline infusion values

TABLE A2 - 3: MEAN (M ±SERUM) POTASSIUM (K^+) VALUES OF UNINFECTED (CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN K^+ VALUES OF UNINFECTED ZEBU CALVES (mMol/l)	MEAN K^+ VALUES OF *T.VIVAX* INFECTED CALVES (mMol/L)	MEAN K^+ VALUES OF *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (mMol/L)
0	4.36 ± 0.07	4.10 ± 0.34	4.55 ± 0.56
3	4.38 ± 0.25	4.05 ± 0.20	4.45 ± 0.03
4	4.37 ± 0.46	4.30 ± 0.12	4.15 ± 0.26
5	4.47 ± 0.24	4.20 ± 0.35	4.26 ± 0.21
6	4.50 ± 0.20	4.30 ± 0.12	4.55 ± 0.34 *4.43 ± 0.21
7	4.45 ± 0.28	3.90 ± 0.06	4.33 ± 0.22 *4.15 ± 0.205
8	4.56 ± 0.22	3.70 ± 0.12	4.26 ± 0.47 *4.00 ± 0.23
9	4.50 ± 0.17	3.65 ± 0.19	4.22 ± 0.27 *4.30 ± 0.28
10	4.43 ± 0.62	4.00 ± 0.00	4.32 ± 0.26 *4.48 ± 0.26
11	4.36 ± 0.18	4.25 ± 0.02	4.00 ± 0.13
12	4.50 ± 0.06	4.40 ± 0.06	4.10 ± 1.17
13	4.43 ± 0.90	4.55 ± 1.44	4.05 ± 0.14

* Post lactose-in-saline infusion values

TABLE A2 - 4: MEAN (M ± SERUM) SERUM BICARBONATE (HCO_3) VALUES OF UNINFECTED (CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES; INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN HCO_3^- VALUES OF UNINFECTED ZEBU CALVES (mMol/l)	MEAN HCO_3^- VALUES OF *T. VIVAX* INFECTED CALVES (mMol/L)	MEAN HCO_3^- VALUES OF *T. VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (mMol/L)
0	23.70± 0.48	24.02 ± 3.12	26.05 ± 2.00
3	24.06 ± 1.16	21.06 ± 4.60	24.5 ± 1.25
4	25.07 ± 1.86	23.50 ± 1.44	24.0 ± 1.15
5	24.33 ±1.51	22.00 ± 2.31	23.5 ± 0.29
6	24.07±1.70	22.00 ± 0.29	24.0 ± 0.00 *24.3 ± 0.96
7	24.67± 0.67	23.00 ± 0.29	22.9 ± 1.65 *23.25 ± 1.79
8	25.07± 1.70	24.56 ± 0.39	25.00 ± 1.47 *24.25 ± 1.68
9	25.61± 1.16	24.60 ± 2.02	23.0 ± 2.06 *22.65 ± 2.22
10	25.67± 1.20	28.60 ± 0.00	25.00 ± 1.91 *24.25 ± 1.92
11	24.67± 2.40	25.66 ± 1.44	27.50 ± 1.55
12	24.67± 2.56	31.00 ± 2.59	24.60 ± 1.15
13	25.07± 1.73	27.56 ± 2.88	26.00 ± 1.68

* Post lactose-in-saline infusion values

TABLE A2 - 5: MEAN (M ± SERUM) SERUM GLUCOSE (GLU) VALUES OF UNINFECTED (CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

III

DAYS POST INFECTION (P.I).	MEAN (GLU) VALUES OF UNINFECTED ZEBU CALVES (mMol/l)	MEAN (GLU) VALUES OF *T. VIVAX* INFECTED CALVES (mMol/L)	MEAN (GLU) VALUES OF *T.VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (mMol/L)
0	3.80± 0.86	3.60 ± 0.67	4.00 ± 2.02
3	3.47 ± 0.17	1.89 ± 0.57	2.35 ± 0.66
4	3.77 ± 0.74	1.65 ± 0.32	1.25 ± 0.29
5	3.83 ±0.15	1.05 ± 0.14	1.58 ± 0.66
6	4.01±2.68	2.37 ± 0.15	4.13 ± 0.86 *3.68 ± 1.50
7	3.82± 0.08	4.12 ± 0.58	3.54 ± 0.99 *4.13 ± 0.37
8	3.65± 2.89	4.85 ± 0.32	3.90 ± 0.75 *3.52 ± 0.58
9	4.10± 0.29	5.62 ± 0.22	3.91 ± 0.39 *3.45 ± 0.66
10	3.79± 2.21	4.33 ± 0.58	2.41 ± 0.56 *2.32 ± 1.74
11	3.83± 0.78	3.80 ± 0.43	2.70 ± 0.80
12	4.53± 2.16	3.81 ± 0.00	2.75 ± 0.94
13	4.23± 1.49	3.16 ± 1.09	2.05 ± 1.20

* Post lactose-in-saline infusion values

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TABLE A2 - 6: MEAN (M ± SERUM) SERUM BILIRUBIN (BIL) VALUES OF UNINFECTED(CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN (BIL) VALUES OF UNINFECTED ZEBU CALVES (μMol/l)	MEAN (BIL) VALUES OF *T.VIVAX* INFECTED CALVES (μMol/L)	MEAN (BIL) VALUES OF *T. VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (μMol/L)
0	6.74± 0.54	6.84 ± 0.54	7.00 ± 1.67
3	7.07 ± 1.67	5.00 ± 0.00	5.00 ± 0.00
4	7.07 ± 1.67	5.00 ± 0.00	10.00 ± 2.04
5	7.07 ±1.67	7.50 ± 1.44	7.50 ± 1.44
6	7.50±3.34	5.00 ± 0.00	7.50 ± 1.44 *6.25 ± 0.72
7	7.80± 6.06	10.00 ± 0.00	8.13 ± 1.65 *10.00 ± 4.08
8	6.97± 1.67	5.00 ± 0.00	7.50 ± 7.50 *7.50 ± 5.00
9	7.07± 1.67	10.00 ± 2.89	6.25 ± 1.32 *5.00 ± 0.00
10	7.06± 1.60	7.50 ± 1.44	6.85 ± 1.86 *6.25 ± 2.50
11	7.06± 1.67	10.00 ± 2.89	10.00 ± 2.04
12	6.00± 0.00	7.50 ± 1.44	7.75 ± 2.50
13	8.03± 3.63	10.00± 2.89	8.75 ± 2.39

* Post lactose-in-saline infusion values

TABLE A2 - 8: MEAN (M ± SERUM) SERUM BLOOD UREA NITROGEN (BUN) VALUES OF UNINFECTED(CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN (BUN)) VALUES OF UNINFECTED ZEBU CALVES (mMol/l)	MEAN (BUN) VALUES OF *T.VIVAX* INFECTED CALVES (mMol/L)	MEAN (BUN) VALUES OF *T. VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (mMol/L)
0	5.66± 0.35	4.850± 0.98	4.80 ± 0.56
3	5.63 ± 2.34	3.85 ± 1.19	2.93 ± 0.21
4	5.63 ± 1.62	5.80± 0.86	3.53 ± 0.56
5	6.00 ±0.78	4.75 ± 0.43	6.25 ± 0.86
6	6.43±1.16	5.17 ± 0.78	5.53 ± 0.81 *5.43 ± 0.88
7	6.03± 0.15	3.75 ± 0.44	3.46 ± 1.19 *3.00 ± 1.41
8	6.17± 1.42	4.40 ± 0.63	2.59 ± 0.17 *2.75 ± 0.29
9	5.89± 1.26	4.50 ± 0.26	3.24 ± 0.53 *3.15 ± 0.404
10	6.00± 2.03	2.60 ± 2.04	3.43 ± 0.52 *3.65 ± 0.85
11	6.26± 0.15	2.90 ± 0.23	3.90 ± 0.74
12	6.16± 1.02	4.35 ± 0.32	3.98 ± 0.31
13	5.98± 1.19	4.45± 0.85	4.88 ± 1.22

* Post lactose-in-saline infusion values

TABLE A2 - 9: MEAN (M ± SERUM) SERUM ASPARTATE AMINOTRANSFERASE (AST) VALUES OF UNINFECTED(CONTROL) ZEBU CALVES, *T.VIVAX* INFECTED ZEBU CALVES AND *T.VIVAX* INFECTED LACTOSE-IN- SALINE INFUSED ZEBU CALVES. (n=4)

DAYS POST INFECTION (P.I).	MEAN (AST)) VALUES OF UNINFECTED ZEBU CALVES (IU/L).	MEAN (AST) VALUES OF *T.VIVAX* INFECTED CALVES (IU/L).	MEAN (AST) VALUES OF *T. VIVAX* INFECTED LACTOSE-IN-SALINE INFUSED ZEBU CALVES (IU/L).
0	52.00 ± 2.10	50.00± 2.10	51.00 ± 3.20
3	50.63± 2.08	38.75± 0.86	38.50 ± 2.14
4	52.00± 6.66	37.60 ± 1.73	40.25 ± 3.47
5	48.07±7.02	44.60± 0.00	52.60 ± 10.87
6	46.60 ±4.57	52.67 ± 10.39	48.56 ±6.18 *40.50 ± 3.73
7	51.07 ± 1.53	46.00± 5.77	32.50 ± 6.08 *34.50 ± 13.08
8	46.86 ± 6.03	42.00 ± 4.62	35.38 ± 4.16 *51.06 ± 3.11
9	48.00 ± 3.28	18.50± 0.87	36.63 ± 2.12 *22.75 ± 7.63
10	56.67 ± 2.62	30.67± 4.16	30.25 ± 5.18 *31.00 ± 3.83
11	48.66 ± 4.26	25.67± 2.88	29.00 ± 3.56
12	52.00 ± 2.38	22.60 ± 2.16	29.60 ± 2.68
13	46.66 ± 5.54	31.56 ± 4.90	29.75 ± 3.90

* Post lactose-in-saline infusion values